DAIRY BACTERIOLOGY

BERNARD W. HAMMER

Paul Arup
May 1929

95271

WILEY AGRICULTURAL SERIES

EDITED BY

J. G. LIPMAN

DAIRY BACTERIOLOGY

THE WILEY AGRICULTURAL SERIES

EDITED BY

J. G. LIPMAN

Dairy Bacteriology.
By BERNARD W. HAMMER, Iowa State College. 473 pages. Ill.

Sugar Cane and Its Culture.
By F. S. EARLE. 256 pages. Ill.

Condensed Milk.
By ATSUSHI MIYAWAKI, Hokkaido Imperial University. 380 pages. Ill.

Green Manuring.
Principles and Practice. By ADRIAN J. PIETERS, Bureau of Plant Industry, U. S. Dept. of Agriculture. 356 pages. Ill.

The Commerce of Agriculture.
A Survey of Agricultural Resources. By FREDERICK A. BUECHEL, Bureau of Agricultural Economics, Washington, D. C. 439 pages. Ill.

Potash.
A Review, Estimate, and Forecast. By J. W. TURRENTINE, Bureau of Soils, U. S. Department of Agriculture. 188 pages. Ill.

Laboratory Experiments in Dairy Chemistry.
By LEROY S. PALMER, University of Minnesota. 86 pages. Ill.

Soil Management.
By FIRMAN E. BEAR, Ohio State University, and Ohio Agricultural Experiment Station. Second Edition, Rewritten. 412 pages. Ill.

Principles of Dairying—Testing and Manufacture.
By H. F. JUDKINS, Eastern Dairies, Inc., Springfield, Mass. 279 pages. Ill.

Genetics in Plant and Animal Improvement.
By DONALD FORSHA JONES, Connecticut Agricultural Experiment Station, New Haven. 568 pages. Ill.

Animal Breeding.
By LAURENCE M. WINTERS, University of Saskatchewan. 309 pages. Ill.

Agricultural Marketing.
By JOHN T. HORNER, Michigan State College. 249 pages. Ill.

Beef Cattle.
By ROSCOE R. SNAPP, University of Illinois. 449 pages. Ill.

Essentials of Systematic Pomology.
By BROOKS D. DRAIN, Massachusetts Agricultural College, Amherst, Mass. 284 pages. Ill.

The Production of Cotton.
By GILBEART H. COLLINGS, Ph.D., Clemson Agricultural College. 256 pages. Ill.

Uniformly bound in cloth. 6 by 9 inches.

DAIRY BACTERIOLOGY

BY

BERNARD W. HAMMER, Ph.D.
Professor of Dairy Bacteriology, Iowa State College, and Chief in Dairy Bacteriology, Iowa Agricultural Experiment Station

NEW YORK
JOHN WILEY & SONS, Inc.
London: CHAPMAN & HALL, Limited
1928

Copyright, 1928
by
Bernard W. Hammer

Printed in U. S. A.

PRESS OF
BRAUNWORTH & CO., INC.
BOOK MANUFACTURERS
BROOKLYN, NEW YORK

PREFACE

The material presented in the following pages represents that included in the beginning course in dairy bacteriology at Iowa State College. This course follows one in general bacteriology and is intended primarily for students in the course in dairy industry but is also required of students in dairy husbandry and is elected by others specializing in bacteriology. Four recitation-lecture periods are held each week for one quarter of about twelve weeks. Assigned readings intended to supplement the material presented and to give the students a more detailed knowledge of important points than the class room permits are also required. The laboratory work consists of three two-hour periods each week and the exercises are selected with the idea of illustrating the more important procedures used in dairy bacteriology. Considerable time is spent on the milk fermentations since a good understanding of these is believed to be fundamental to the practical knowledge of dairy bacteriology which is so essential to the technical dairyman. Some of the organisms studied are isolated by the students themselves from milk showing various changes. This illustrates the distribution of the organisms studied better than can be done in any other way and also adds interest to the work; the continued study of supplied cultures tends to give the students the impression that bacteria exist mainly in laboratory cultures.

The relative importance, from the standpoint of the class room, of the various phases of dairy bacteriology is a question on which considerable diversity of opinion is to be expected. The proportion maintained in the following presentation has been found to be satisfactory under the conditions existing at Iowa State College and satisfactorily to relate dairy bacteriology to the courses that follow it.

Material on the standard methods for the bacteriological examination of milk is not included because it is so readily available elsewhere in a form adapted to laboratory use. For the most part, descriptions of organisms are omitted because brief descriptions are included in books dealing with determinative bacteriology and detailed descriptions can be best studied in the original reports.

B. W. HAMMER.

Dairy Industry Building,
Ames, Iowa.

Digitized by the Internet Archive
in 2022 with funding from
Kahle/Austin Foundation

https://archive.org/details/dairybacteriolog0000bern

TABLE OF CONTENTS

CHAPTER PAGE

INTRODUCTION . . . xi

I. BACTERIAL COUNTS ON MILK . . . 1
  Routine counts . . . 2
  Research counts . . . 16

II. MILK FERMENTATIONS . . . 18
  The lactic acid fermentation . . . 20
  The ropy or slimy fermentation . . . 42
  The gassy fermentation . . . 51
  The sweet curdling fermentation . . . 57
  Proteolysis . . . 60
  The flavor and aroma fermentations . . . 62
  The color fermentations . . . 69
  The volatile acid fermentations . . . 72
  Numbers of micro-organisms required to produce definite changes in milk . . . 74
  Characteristics of milk abnormalities due to the growth of micro-organisms . . . 76

III. CONTAMINATION OF MILK AND CREAM . . . 78
  Contamination from the interior of the udder . . . 78
  Contamination from external sources . . . 87
    Contamination on the producing farms . . . 87
      The stable air . . . 87
      The utensils . . . 91
      The coat of the animal . . . 97
      The milker . . . 100
      Miscellaneous sources . . . 100
    Contamination subsequent to production . . . 101

IV. REDUCING THE CONTAMINATION OF MILK . . . 107
  Methods of reducing the contamination of milk on producing farms 107
  Methods of reducing the contamination of milk and cream in dairy plants . . . 124
  Chemical sterilization of dairy equipment . . . 126

V. GROWTH OF MICRO-ORGANISMS IN MILK AND CREAM . . . 129

VI. BODY CELLS IN MILK . . . 143

CHAPTER PAGE
VII. SPREAD OF DISEASES THROUGH MILK AND ITS DERIVATIVES . . . 153
Group 1. Diseases in which the infectious material comes primarily from the producing animals . . . 156
Tuberculosis 156
Malta fever 170
Contagious abortion 173
Foot and mouth disease 178
Johne's disease 179
Anthrax 180
Actinomycosis 181
Rabies 181
Udder inflammations 181
Milk sickness or trembles 184
Group 2. Diseases in which the infectious material comes from man directly or indirectly 186
Typhoid fever 191
Paratyphoid fever 207
Dysentery and diarrhea 209
Septic sore throat 210
Scarlet fever 218
Diphtheria 221
Poliomyelitis 226
Cholera 228
Infantile diarrhea 228
Summary of the contamination of dairy products with pathogens . 232
Preventing the dissemination of diseases through dairy products . 233
Poisoning from dairy products 234

VIII. PRESERVATION OF MILK AND CREAM 237
Removal of contained micro-organisms 237
Straining or filtering 238
Separation 242
Clarification 244
Prevention of the growth of organisms 250
Low temperatures 250
Preservatives 251
Destruction of micro-organisms 253
Advantages of pasteurization 271
Objections that have been raised to pasteurization 274
The electrical treatment of milk 277
Effect of ultra-violet rays on bacteria in milk 279

IX. MILK ENZYMES 280

X. BACTERIOLOGY OF EVAPORATED, SWEETENED CONDENSED AND POWDERED MILK 287
Bacteriology of evaporated milk 287
Bacteriology of sweetened condensed milk 294
Bacteriology of milk powder 299

CHAPTER PAGE

XI. BACTERIOLOGY OF ICE CREAM 304
- Sources of bacteria in ice cream 307
  - Bacteria in the material used 307
  - Contamination incident to the manufacture and handling . . 310
- Changes in the numbers of bacteria during the manufacture of ice cream 312
- Additional considerations 318

XII. BACTERIOLOGY OF BUTTER CULTURES 320
- Propagation of butter cultures 324
- Judging butter cultures 330
- Influence of various factors on butter cultures 333
- Organisms normally present in butter cultures 336
- Development of butter cultures 345

XIII. BACTERIOLOGY OF FERMENTED MILKS 348

XIV. BACTERIOLOGY OF BUTTER 354
- Sources of the flavor in butter 365
- Factors involved in butter deterioration 367
- General types of changes that occur during butter deterioration . 379
- Specific flavor defects of butter 380
  - Flavor defects primarily due to unsatisfactory methods in the plant 381
  - Flavor defects primarily due to off flavors in the cream . . 382
  - Flavor defects that develop primarily during the holding of the butter 385
- Color changes in butter due to micro-organisms 391

XV. BACTERIOLOGY OF CHEESE 401
- Bacteriology of cheddar cheese 405
  - Defects of cheddar cheese due to micro-organisms 418
- Bacteriology of Swiss or Emmental cheese 426
- Bacteriology of Roquefort cheese 431
- Bacteriology of Camembert cheese 436
- Bacteriology of cottage cheese 441

XVI. TESTS FOR THE QUALITY OF MILK AND CREAM 445
- Tests involving the detection of the products of the growth of micro-organisms 445
  - Examination for flavor and aroma 445
  - Titration for an increased acidity 446
  - Alcohol test 447
- Tests involving the rate at which changes are produced by the contained micro-organisms 449
  - Keeping quality tests 449
  - Keeping quality in the presence of an indicator 449
  - Changes produced in pH of bouillon by organisms in added milk 450
  - Methylene blue reduction test 451

CHAPTER PAGE
Tests involving the presence of certain types of micro-organisms . 455
  Wisconsin curd test 455
  Anaerobic spore test 456
  Tests for Escherichia-Aerobacter organisms 457
  Tests for streptococci 458
  Tests for specific pathogens 459

INDEX 461

INTRODUCTION

IMPORTANCE OF DAIRY BACTERIOLOGY

The need for a knowledge of the bacteriology of dairy products is shown by three distinct considerations as follows:

1. The spread of diseases through dairy products.

   Investigations extending over many years have proved that diseases in both man and animals have sometimes been caused by the spread of pathogenic organisms through milk and its derivatives. While the presence of such organisms is not peculiar to food materials from the dairy but occurs with products from a variety of sources, it is especially serious with the former because they are so often consumed without being heated and thus without the harmful organisms being destroyed. Accordingly, a knowledge of the sources of the pathogenic organisms in dairy products, the conditions under which they may grow and the methods of keeping them out or destroying them when they gain entrance, is essential. Such knowledge is important to the producer or manufacturer of dairy products, because of the responsibility of selling only safe foods, and it is of extreme importance to the inspector of dairy plants and dairy products since inspection is intended to represent the final protection to the consumer. While the producer or manufacturer and the inspector are supposed to look after the consumer's interests, it is desirable that the consumer be sufficiently informed to decide whether or not a particular system of handling a dairy product is satisfactory. In the same way, the farmer or other individual using milk or milk products for the feeding of animals should have the knowledge that will make possible a reliable decision as to the possibility of such materials introducing diseases into his herds and flocks.

2. The deterioration and spoilage of milk and its derivatives.

   Many micro-organisms, as a result of their growth, cause undesirable changes in milk and its derivatives and are, accordingly, responsible for deterioration and spoilage. The producer of milk and cream should know the sources of the organisms causing rapid changes in these materials, the conditions favoring their growth and the methods of eliminating them or preventing their activity, since the income secured is, to a considerable extent, dependent on the quality of the products supplied. The manufacturer of milk derivatives must contend with problems similar to those of the producer of milk and cream and additional ones also, since butter, cheeses, etc., are frequently stored for considerable periods, during which there may be unusual opportunities for a decrease in quality. The problem of deterioration and spoilage is especially important with the cheeses requiring ripening since conditions must be favorable for the growth of certain desirable micro-organisms and these may also allow the development of undesirable types.

3. The development of desirable types of micro-organisms in certain dairy products.

The pleasing flavor and aroma of some of the dairy products, such as butter, cheeses and fermented milks, and the desired texture of many types of cheese are largely due to the development of certain micro-organisms during the manufacturing and ripening processes. An understanding of the action of these organisms, the conditions favoring their growth, etc., is important and very necessary if products having a uniformly high quality are to be put on the market. The manufacturing methods used for many dairy products were developed empirically before the role of micro-organisms was understood, but various valuable improvements have resulted from a knowledge of the action of the important groups and progress along this line will undoubtedly continue.

DAIRY BACTERIOLOGY

CHAPTER I

BACTERIAL COUNTS ON MILK

Investigations of the numbers of bacteria in milk quickly followed the development of methods applicable to such determinations. The early studies were apparently intended mainly to aid in satisfying an interest in the bacterial content of various materials known to contain these organisms, but soon the value of bacterial counts as an indication of the general conditions of production and handling and of the keeping qualities of milk became evident and counts began to be used in securing information along these lines. Determinations of the numbers of bacteria have also come into extensive use in investigations of the role of various organisms in both the desirable and undesirable changes occurring in dairy products.

Types of bacterial counts. There are two general types of bacterial counts used with milk and certain of its derivatives; these are referred to as *routine* and *research* counts. Their objects are very different. The routine counts are used in the general control of milk supplies and in the grading of milk, while the research counts are employed where accuracy under specific conditions is the principal consideration. With routine counts the results must be secured cheaply and as quickly as possible, while with research counts expense and time are not such important factors. Definitely standardized methods are necessary with routine counts so that the results obtained in different laboratories may be compared and so that the significance of various counts may be interpreted on the basis of the general experiences in milk control work. It is not necessary that routine counts give the actual numbers of bacteria present in a sample, but rather that a representative portion be determined. The bacterial limits set by ordinances and regulations quite regularly take into account the fact that the routine methods of examination do not necessarily give as high counts as might be obtained by special methods. Counts for research purposes may be secured under widely differing conditions, depending on the particular object in view, but it is important that the exact procedure be clearly given in any statement of the results obtained.

ROUTINE COUNTS

Significance. At the present time routine bacterial counts are widely used in attempting to arrive at the general sanitary quality of milk. If milk is produced under dirty conditions, if it is kept warm, or if it is held even at satisfactory storage temperatures for an extended period, high bacterial counts result. Occasionally, certain udder conditions of the cow may also be responsible for large numbers of bacteria. A routine bacterial count on a sample of milk may, accordingly, be considered as giving information along four lines as follows:

1. Care used in the production.

   The care used in the production of milk, especially with reference to such factors as the condition and type of the utensils and the cleanliness of the bodies of the cows, is quickly reflected in the bacterial count of the milk at the time of milking. However, even with very filthy conditions of production the counts are not excessive at the time the milk is drawn; they only rarely exceed 1,000,000 per cc. and commonly fall below 500,000. It is the multiplication of the organisms gaining entrance to the milk, rather than the contamination, that is responsible for the very high counts sometimes secured.

2. Temperature of holding.

   The rate of multiplication of bacteria in milk is influenced more by the temperature of holding than by any other factor. At favorable temperatures growth is surprisingly rapid and excessive numbers of organisms quickly develop. Very high bacterial counts on milk are undoubtedly more often due to holding temperatures that are especially favorable for the multiplication of bacteria than to any other cause. The influence of a particular temperature on the rate of growth varies widely with different organisms. Another factor of importance in connection with the temperature effect is the history of the milk; fresh milk held at 10° C. (50° F.) is not likely to show any significant increase in the numbers of bacteria in 24 hours, while milk that has been aged for some time may show considerable growth under the same conditions.

3. Time of holding.

The time of holding is another important factor influencing the multiplication of bacteria in milk and one that is naturally very closely related to the temperature of holding. As short a period as one hour may account for an increase in the number of bacteria present in milk, if the holding temperature is very suitable for growth, while longer and longer periods are required for significant increases as the temperature becomes less and less favorable. Temperatures that satisfactorily check bacterial development for 24 hours may not do so over more extended periods. If the holding period is sufficiently long the multiplication of bacteria occurs in naturally contaminated milk at all the usual temperatures above the freezing point and, accordingly, even temperatures approximating 0° C. (32° F.) do not justify excessive holding, although they prevent the multiplication of bacteria for a considerable time.

4. Condition of the udder.

High bacterial counts on milk may be obtained as a result of excessive numbers of bacteria being present in the milk at the time it leaves the udder. Frequently, such milk comes from udders showing an infection of some sort but it may also come from udders that are apparently normal. There is no close correlation between infected udders and high counts on the milk at the time it is drawn and in some instances milk coming from such udders has an unusually low bacterial count.

The information given by routine bacterial counts along the four lines just considered is suggestive rather than definite. When a high count is secured it is usually more or less difficult to decide which of the various factors is responsible, although laboratory experience often makes it possible to give a reasonably accurate opinion; a variety of bacterial types suggests extensive contamination, many colonies resembling the common lactic acid organisms indicate growth at temperatures higher than those usually advocated for holding milk, etc. Commonly, high counts are due to a combination of factors and, when this is the case, the determination of the cause is much more complicated than when only one factor is involved. While the history of a sample of milk, as determined by the enumeration of the contained bacteria, is not as satisfactory as might be desired, defective methods of production and handling can be detected and, accordingly, bacterial counts are almost indispensable in the proper control and regulation of milk supplies.

Counts on pasteurized milk. Bacterial counts are very valuable in the supervision of supplies of pasteurized milk since they aid materially in deciding on the efficiency of both the pasteurization and the handling of the milk after heating. While pasteurization temperature and time records are important, there is no assurance that all of the milk has been heated under the recorded conditions and, moreover, the value of efficient pasteurization may be more than counter-balanced by carelessness with the milk subsequent to this treatment. Usually counts are made on the raw milk also so that a product of a poor sanitary quality originally can be detected; these preliminary counts are particularly necessary when there is a tendency to use pasteurization as a means of marketing milk that would otherwise be unsuitable for this purpose.

Value of milk inspection. Bacterial counts do not give all the information desired in the careful supervision of milk supplies and the inspection of the farms, the milk plants and the methods of production, transportation and handling, furnishes valuable additional data, especially with reference to the exact location of defects in the methods or equipment. In addition to supplementing each other, inspection and bacterial analyses serve to check one another. This is of particular importance when high bacterial counts accompany reports from the inspection service indicating satisfactory conditions; if this occurs, the bacteriological results may be used to direct the inspection in an effort to locate the cause of the trouble.

The relatively low cost of bacterial counts is an important consideration and it is quite generally recognized that, for the expense involved, bacterial counts give more information regarding the methods of producing and handling milk than any other procedure that can be used. At the present time bacterial counts are extensively employed by milk companies and distributors, in addition to health departments and other supervising agencies. A bacteriological laboratory is an important part of many modern dairy plants and, in some of these, large numbers of bacterial counts are run on the milk as it comes from the producers; in certain instances a premium is paid for milk of a high sanitary quality as shown by these counts.

An examination of the milk is also commonly made at various stages in its handling in the plant in order to check the methods followed.

Criticism of routine counts. Bacterial counts have been criticized as being of little value in determining the safety of a milk supply since the great majority of the organisms present in milk are harmless. *Streptococcus lactis* is the most common type in milk having a high bacterial count and it is consumed in large numbers in such products as buttermilk, cottage cheese, butter, etc., that are regarded as valuable foods. However, it must be recognized that the conditions which admit of the entrance of organisms, or of the growth of those that do gain entrance, are the conditions most likely to result in the introduction or multiplication of pathogenic types so that, on the average, the lower the count the less the danger of harmful organisms being present. A high bacterial count is no indication that milk is harmful and, on the other hand, a low count is no guarantee that milk is safe, but the conditions of production and handling that give high counts are more likely to result in the presence of harmful organisms than conditions yielding low counts. There is, of course, a great difference in the importance of organisms from various sources, a few getting into milk from an infected human throat, for example, being much more objectionable than a larger number from the flanks of the cows or from the utensils, and it must be admitted that total bacterial counts do not take this into consideration. However, they are an indication of the care used in the production and handling of milk and careful methods are necessary in providing a satisfactory milk supply. The ideal type of examination would be one which involves a determination of the dangers from the various organisms present and, when such a method of examination is devised, it will undoubtedly supersede bacterial counts. There are, however, many difficulties involved in such a determination and it seems inadvisable to discard the present method, with its admitted defects, until something better is available with which to replace it.

In some instances it has been found that milk pasteurized at satisfactory exposures has a high count immediately after heating, due to the multiplication of certain bacteria in the operating pasteurization equipment. These organisms have unusually high growth temperatures and develop under conditions which destroy many types of organisms. They often form tiny colonies on agar plates that are sometimes referred to as ''pin-point colonies''; these make the counting difficult because they are not readily recognized as colonies and must often be identified under a lens. High counts on milk, immediately after pasteurization at satisfactory exposures, have been advanced as an objection to the use of counts in arriving at the quality of milk since they give the impression that the milk has been carelessly handled. The serious development of the heat resistant organisms in pasteurization equipment is, however, only occasionally encountered and should not discredit the whole system of bacterial counts on pasteurized milk when so much valuable information may be secured with it; moreover, when there is a development of organisms in pasteurizing equipment that is being operated over rather extended periods, this may be effectively controlled by an occasional cleaning between runs.

Methods used for routine counts. There are two methods commonly used in the routine counting of the bacteria in milk—the plate method or macroscopic colony count and the microscopic count or direct count or Breed method—both of which have wide fields of usefulness. A third method, the microscopic colony count or Frost method, has also been devised but is employed much less extensively than the other two. The procedures used with these methods are described in detail in "Standard Methods of Milk Analysis."[1]

The plate count. The introduction of liquefiable solid media afforded a means of determining the numbers of bacteria in various materials by mixing a given weight or volume with a fluid medium, allowing this to solidify and then counting the number of colonies developing on incubation. This procedure has been widely used with various materials and has found an extensive application in the examination of milk.

The medium used for plating milk, the temperature and time of incubation and other variations in the method have an influence on the number of colonies developing, through their general effect on the growth of organisms. Recognizing the necessity of standard methods for the bacteriological examination of milk, if the results obtained in different laboratories are to be directly comparable, the American Public Health Association appointed a committee in 1905 to study the various methods used and to recommend a uniform procedure. Preliminary reports were made, a complete report was published in 1910 and additional reports have followed from time to time. The more recent reports have represented the ideas of various organizations interested in milk supervision.

Objections to the plate count. Certain objections have been raised to the routine plate method of determining the numbers of bacteria as a means of controlling milk supplies, the more important of which are the following:

---

[1] American Public Health Assn. 5th ed. 1927.

1. Pathogenic organisms are not detected.

   Because one of the very important reasons for controlling a milk supply is to prevent the spread of diseases through it, the failure of the counts to detect pathogens constitutes a definite defect. These organisms are not detected because certain of them (for example, *Mycobacterium tuberculosis*) do not grow under the conditions provided and because those that do grow (for example, *Eberthella typhosa*) can not be distinguished from non-pathogenic types by the colony appearance. If a rapid and accurate routine method for the determination of pathogens in milk is ever devised, it will undoubtedly soon supplant routine bacterial counts to a large extent.

2. Counts are not accurate.

   The counts obtained by the plate method are not accurate because of a number of factors. Many of the organisms present in milk do not find the conditions provided favorable for their development. Although the counts obtained with certain agar preparations, such as meat infusion agar, are often higher than those secured with standard agar, none of the known media provide satisfactory nutrients for all the organisms found in milk. An incubation temperature of 37° C. (98.6° F.) is unsuitable for some of the organisms and certain others grow so slowly that the colonies are not recognizable when the incubation is complete. Another reason why plate counts are always inaccurate is that many of the colonies come from a group of cells instead of from one cell, as is assumed in the calculation of the number of bacteria per cubic centimeter. It is, accordingly, evident that even with very careful technique the results obtained by the plate method are always low; the extent of the discrepancy is dependent mainly on the types of bacteria present in the milk. The fact that the results are low is not of any great consequence in the control of milk supplies since an allowance is made for this in the establishment of bacterial standards. The method used should, however, give a fairly constant percentage of the total numbers of bacteria present.

   In some instances large discrepancies [2, 3 and 4] have oc-

---

[2] Pub. Health Rpts. 30. P. 2349. 1915.

[3] Am. Jr. Pub. Health. 8. P. 913. 1918.

[4] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 75. 1920.

curred in the results obtained by various laboratories on milk from the same lot. These may be due to variations in the media used, in the time between diluting the milk and pouring the plates, in the methods of incubating the plates and in other factors, all of which are of importance and may need additional standardization. Differences in the results obtained by persons working in the same laboratory have also been reported. It should be recognized, however, that often two or more counts which show considerable variation lead to the same general conclusion with reference to the care used in the production and handling of the milk so that this variation is of little practical importance. In many trials the agreement between the results obtained in different laboratories is surprisingly close when consideration is given to the fact that the growth and development of microscopic organisms is involved.

3. Specific information regarding species is not furnished.

The colony appearance, with most organisms, gives little knowledge as to the species. If a classification of the organisms could be made from the colonies, valuable information regarding the milk could undoubtedly be obtained. However, the failure to secure definite information regarding species can not be considered a serious objection to the plate method when it is recognized that the counts are made primarily with the idea of securing general information on the conditions of production and handling of the milk.

4. Time required before the results are available is too long.

Because of the time required for the incubation of the plates, data on the number of bacteria contained in a sample of milk are ordinarily not available until the milk has been consumed. The quality of a milk supply, however, can not be justly determined by one bacterial count and it is only when the results of a series of counts are available that a fair idea of the character of a supply can be formed. A single high count does not justify the exclusion of the lot of milk on which it was obtained, if the counts secured on the preceding lots from the same source are satisfactory; it does, of course, indicate an undesirable condition that should be located and corrected at the earliest possible moment. When the real significance of a single high count is realized, it is evident that the consumption of milk before the counts on it are available does not constitute an important objection to the plate method.

The microscopic count. Methods of counting bacteria in various materials by means of a microscope have been employed since the early days of bacteriology and the idea was eventually applied to milk. The Breed method, which is the one widely used in the United States at the present time, involves the spreading of 0.01 cc. of milk, measured with a capillary pipette, over an area of one square centimeter on a glass slide and, after removal of the fat, the fixing, staining and examination of the resulting film. Newman[5] has developed a procedure which permits the removal of the fat and the fixing and staining of the film with one reagent.

Some of the early attempts to count the bacteria in milk by means of a microscope consisted of preparing a film from the material thrown out of the milk with a centrifuge; it seems impossible to recover all of the organisms in this way and the methods employing the principle are not widely used at present.

Advantages of the microscopic count. The microscopic count has certain advantages over the plate count, the more important of which are the following:

1. Results are quickly available.

   With the microscopic method a count may be obtained in as short a time as 15 or 20 minutes, provided the microscope has been standardized and the stain and other reagents prepared; this is particularly advantageous in connection with samples brought to laboratories by producers since, as a rule, such persons are anxious for the results at once and are often interested in actually seeing the bacteria present in the milk.

2. Less work is required.

   The number of samples that can be examined in a given time with the microscopic method is considerably larger than the number that can be examined with the plate method, due mainly to the fact that the preparation of the medium and the dilution bottles, the washing and sterilization of the glassware, etc., are not involved. If only approximate microscopic counts are desired, for example for the purpose of grading milk, they can be secured very rapidly. Breed and Brew[6] reported that in the examination and classification into three grades of 11,851 cans of milk by the microscopic method, the person who did the work found it possible to handle from 50 to 75 samples each working day.

---

[5] Monthly Bul. Dept. Agr. Cal. 16. P. 1. Jan. 1927.

[6] N. Y. (Geneva) Agr. Expt. Sta. Bul. 443. 1917.

3. Less apparatus is required.

The amount of apparatus necessary with the microscopic method is comparatively small, a microscope being the only expensive item. When the plate method is used the investment in incubators, sterilizers, glassware, etc., is quite large.

4. Less expensive.

Since the microscopic method requires less work and apparatus than the plate method, the expense per sample is also less. The lowest possible cost is advisable, if bacterial counts are to be extensively used, because the funds available are often rather limited. When only a small number of bacterial counts are made on a given milk supply the conclusions drawn are likely to be open to criticism.

5. Microscopic preparations give a permanent record.

The microscopic preparations used in making bacterial counts can be kept and examined whenever, within a reasonable time, a question arises with reference to the counts. Permanent records are especially important in connection with certain points, such as the morphology of the organisms present, that are difficult to report and not easily included in a statement of the results.

6. Some idea of the species present can be secured.

While no very definite information regarding species can be obtained from microscopic observations alone, some idea of the general types of bacteria present can be secured by the person familiar with the organisms common in milk. A predominance of the ordinary lactic acid bacteria is rather easily determined from the microscopic preparations and some of the organisms coming from infected udders can be recognized with considerable accuracy. The information obtained on the types of organisms is frequently very valuable in helping to determine the cause of high bacterial counts on certain samples.

7. The number of body cells present can be determined.

The stained preparations employed in the microscopic method permit the counting of the leucocytes and other body cells present in milk and unusually large numbers are evident from a very superficial examination. While the significance of these cells, even in rather large numbers, may be questioned, excessive counts indicate abnormal udder conditions, particularly when they are associated with large numbers of streptococci. The analyst experienced in the microscopic method can frequently detect abnormal udders by the microscopic picture alone.

8. Counts may be made so as to more nearly represent the actual numbers of organisms.

   The plate method is, in reality, a count of the colonies which develop on standard agar while with the microscopic method a count is made of the number of stained bacterial cells present. Although undoubtedly certain organisms, such as the acid fast forms, do not stain and some cells that do stain are dead, these usually make up only a small percentage of the total number of bacteria in raw milk. The microscopic method, accordingly, may be used to secure counts that represent the actual numbers of organisms present more closely than do the plate counts. By counting clumps, chains, pairs and single cells as units, the microscopic counts can be made to more nearly approximate the plate counts, although they are commonly higher because some of the organisms do not develop on the standard agar plates. The difference between the microscopic and plate results is largely determined by the species of organisms present in the particular sample.

9. Preservatives may be used to prevent growth in samples.

   Milk samples intended for microscopic examination may be held for short periods with preservatives, such as formaldehyde, because the organisms need not be kept in a living condition. Samples so treated can not be held for very long, however, since the organisms killed by the preservative soon begin to autolyze and this seriously interferes with their staining. If the preserved samples are allowed to stand until the cream is very compact, difficulty is experienced in redistributing it.

Disadvantages of the microscopic count. In addition to its advantages the microscopic method has certain disadvantages when compared to the plate method. The more serious of these are as follows:

1. Less generally useful for the examination of pasteurized milk.

The bacteria in raw milk and some of those recently killed by pasteurization are not readily distinguished when subjected to the technique of the microscopic method and, accordingly, the microscopic counts secured on pasteurized milk can not be assumed to represent only living cells. This seriously limits the usefulness of the method in the supervision of city supplies, since at present all of the larger cities have a considerable portion of the milk pasteurized, and in the checking of operations in pasteurizing plants. Hastings and Davenport[7] noted that as a result of pasteurization at 62.8° C. (145° F.) for 30 minutes there was a decrease in the numbers of bacteria detected by the microscopic method which, in some instances, was very large. The decrease was often more after six hours storage at 10° C. (50° F.) than immediately after heating and, occasionally, there was a further decrease after 24 hours storage. These investigators also found that the pasteurization of low count milk inoculated with pure cultures commonly gave results similar to naturally inoculated milk, although with some of the species there was no apparent change in the staining or the number of cells.

A microscopic count in addition to a plate count on a sample of pasteurized milk may supply valuable information with reference to the number of bacteria present before heating and also help to explain the cause of unsatisfactory pasteurization by showing the general morphologic types of organisms present.

A method of staining preparations made from pasteurized milk so that the living and dead bacteria can be differentiated has been developed by Beattie.[8]

2. Less generally useful for the examination of low count milk.

The factor used in converting the average number of bacteria per field to the number per cubic centimeter varies with the microscope adjustments but usually runs from 300,000 to 600,000. Due to the large conversion factor, a small error in the average number of bacteria per field means a considerable error in the calculated number per cubic centimeter. Errors in the average number per field are not likely to be of significance when considerable numbers are present in the milk but may cause more serious inaccuracies in low count milk with which many fields show no bacteria. In some instances the microscopic method has been satisfactorily used on very low count milk but it is a laborious procedure because of the large number of fields that must be counted.

---

[7] Jr. Dairy Sc. 3. P. 494. 1920.

[8] Am. Jr. Pub. Health. 17. P. 1031. 1927.

Comparison of plate and microscopic counts. In a comparison of the microscopic method and the plate method of counting bacteria in milk, Brew [9] found that with samples showing a plate count under 10,000 per cc. the microscopic method gave individual cell counts approximately 44 times as high as the plate counts while, when the groups and isolated cells were counted as units, the counts were approximately 17 times as high; with samples showing a plate count of about 1,000,000 per cc., the microscopic method gave individual cell counts approximately 5 times as high as the plate counts while, when the groups and isolated cells were counted as units, the results were slightly lower than with the plate method. From these results it is evident that the differences between the microscopic counts and the plate counts are considerably greater on low count samples than on high count samples. This is, in part, due to the fact that in low count milk the organisms are mainly those that have gotten in from various sources and include types which will not grow on the agar used for plating while, in high count milk, the organisms largely represent those that have grown in milk, most of which are capable of growth on standard agar. There is a tendency also for a greater percentage of the bacteria to be in clumps in low count milk than in a high count product.

Robertson [10] found that the most frequent ratio between the results of microscopic counts and those of plate counts, when the plates were incubated two days at 37° C. (98.6° F.), was approximately four to one; this is the ratio recognized in "Standard Methods of Milk Analysis" and should be used in calculating the results of one type of count in terms of the other. The average size of the clumps of bacteria in milk undoubtedly varies with the species present and also with the conditions under which growth occurs. Baker, Brew, and Conn,[11] in their study of the relation between lactic acid production and bacterial growth in the souring of milk, noted that the average size of the groups of lactic acid organisms in milk was 1.8 individuals. Whiting[12] found that the average size of the clumps of bacteria in 3,480 samples of market milk from the Geneva, New York, supply was 11.1 individuals while in 150 samples from the Ithaca, New York, supply, it was 4.1 individuals; the word clump was used to refer to isolated cells as well as groups.

---

[9] N. Y. (Geneva) Agr. Expt. Sta. Bul. 373. 1914.

[10] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 86. 1921.

[11] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 74. 1919.

The microscopic colony count. In the microscopic colony count, milk is mixed with agar on a sterile glass slide and the mixture spread over a definite area. After hardening, the preparation is incubated in a moist chamber for a number of hours and quickly and thoroughly dried at a temperature a few degrees below the boiling point of water. It is then stained, the colonies per microscopic field determined and the results calculated to a cubic centimeter basis.

Frost[13] considers that the important points with reference to the method are the following:

1. It is rapid, not over seven or eight hours being required.
2. It is reliable in that failure due to spreaders or improper dilutions is unlikely.
3. A relatively large amount of milk, usually 0.25 cc., is studied.
4. A permanent record of the count is provided.
5. Only a small amount of glassware and media is required.

Bacterial standards. The use of bacterial counts for the control of milk supplies has resulted in the establishment of bacterial standards. The New York City Department of Health attempted a standard of 1,000,000 per cc. in 1900, but it was found practically impossible to enforce, at that time, because of the intricacy of the milk supply. In 1905, the Boston Board of Health legalized a standard of 500,000 per cc. At present a large number of cities have bacterial standards for milk; some of them have the same standard as Boston, while others have standards that are more suited to the conditions under which their supplies are produced and handled. In some instances dual standards, which involve one limit for the warm months and another for the cold months, have been formulated; this is simply a recognition of the fact that it is more difficult to keep down the bacterial content of milk in summer than it is in winter.

A lack of uniformity in the milk standards of different cities is to be expected because of the varied conditions under which the different supplies are produced. A city whose supply comes only a short distance often has more rigid requirements than one getting milk from a distant milk shed. While it would be difficult to enforce the same standard in all localities, there is a certain advantage in uniformity, particularly from the standpoint of the producers who may be shipping to one city at one time and to a different city at another.

---

[12] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 98. 1923.

[13] Jr. Bact. 2. P. 567. 1917.

The following examples illustrate the bacterial limits that may be used for milk of various grades:

Proposal of New York Milk Committee:[14]

- Grade A milk, raw and pasteurized milk included.
  - Raw milk: 100,000 bacteria per cc.
  - Pasteurized milk: 200,000 bacteria per cc. before and 10,000 after pasteurization.
- Grade B milk, pasteurized milk only.
  - 1,000,000 bacteria per cc. before and 50,000 after pasteurization.
- Grade C milk, pasteurized milk only.
  - Over 1,000,000 bacteria per cc. before and 50,000 after pasteurization.

Proposal of United States Public Health Service Standard Milk Ordinance:[15]

Grade A milk, raw	50,000 per cc.
Grade B milk, raw	200,000 per cc.
Grade C milk, raw	1,000,000 per cc.
Grade D milk, raw	5,000,000 per cc.

Grade A milk, pasteurized; either grade A or B raw with 50,000 per cc. after pasteurization.
Grade B milk, pasteurized; either grade A, B or C raw with 100,000 per cc. after pasteurization.
Grade C milk, pasteurized; milk not meeting the requirements of grade B pasteurized; 500,000 per cc. after pasteurization.

In addition to the bacterial limits set for the various grades of milk there are commonly other requirements covering the condition of the cows, barns, milk rooms and utensils, as well as the methods used in producing and handling the milk.

Special grades of milk have bacterial limits lower than those given above. The common standard for certified milk is 10,000 per cc. The standard for cream is often higher than that for milk of the same grade since considerable numbers of organisms may be carried into the cream by the clumps of fat globules.

[14] Pub. Health Rpts. Reprint 141. 1913.

[15] Pub. Health Rpts. Reprint 1099. 1926.

There is a distinct disadvantage in having a number of grades of milk in a city since this greatly complicates the enforcement of regulations covering the milk supply and tends to encourage the substitution of one grade for another. While special grades of milk may be advisable for special purposes the bulk of the supply is often advantageously limited to one grade.

In the establishment of a bacterial standard for milk it is necessary to state whether it refers to the plate count or the microscopic count because of the difference in the results secured by the two methods. The early standards largely referred to the plate count since, at the time of their establishment, this was the common method employed. More recently the increased use of the microscopic count has made necessary the formulation of requirements based on it; this has been the case with the standards used by health departments, while the development of methods of paying for milk on the basis of its bacterial count has resulted in such standards also being developed by milk companies.

In general, cities accompany their attempts to enforce bacterial standards with campaigns of education and efforts are made to help the producers and dealers locate and overcome their difficulties. Prosecutions should be rather uncommon and only resorted to when it becomes evident that the vendor is persistently careless or irresponsible. It must not be forgotten that bacteriological examinations of milk constitute a very severe test and that occasional high counts are likely to occur with milk supplied by the most careful producers.

RESEARCH COUNTS

Both the plate method and the microscopic method of counting bacteria are widely used in experimental work, the one that is adopted depending on the object to be accomplished. Often the two methods are used synchronously.

When using the plate method in investigational work it is generally desirable to have the counts as accurate as possible and, accordingly, special media and prolonged periods of incubation are often employed. Frequently, when some particular group of organisms is being studied, a medium or incubation temperature that is especially favorable for it can be adopted.

Beef infusion agar is a very satisfactory medium for the growth of many of the bacteria present in dairy products and, with certain of them, gives considerably larger colonies than beef extract agar.

It is particularly valuable with some of the organisms regularly giving comparatively small colonies, such as *Streptococcus lactis.* Whey agar is useful with the bacteria present in sour milk and cream and with many yeasts and molds, but it is unsatisfactory for certain species finding their way into milk and often gives low total counts when used on sweet milk. Sherman [16] noted that the addition of lactose to beef extract agar increased both the number and size of the colonies developing on plates poured with milk and incubated 48 hours at 37° C. (98.6° F.). Supplee, Whiting and Downs,[17] however, found that with these incubation conditions lactose agar had few if any advantages over plain agar while dextrose agar had distinct advantages over both.

Media which permit of a differentiation of the colonies developing are very useful in work on dairy products. Litmus lactose gelatin was extensively employed at one time but the rapid liquefaction of the medium that occurs with some organisms was found to limit its usefulness. Ayers [18] suggested a casein agar with which the peptonizers as well as the total numbers of bacteria could be counted and later Ayers and Mudge [19] proposed various milk powder agar media that make possible the differentiation of the colonies into several types.

Incubation at 21° C. (69.8° F.) or 30° C. (86° F.) for several days commonly gives higher counts on dairy products than incubation at 37° C. (98.6° F.) for 48 hours and can ordinarily be used in research work since it is not necessary that results be secured quickly. Among the organisms present in milk and its derivatives, species that fail to grow at 37° C. are occasionally encountered.

In some instances it may be advisable to restrain the growth of certain organisms so that the types being investigated can be more easily counted or isolated. This is done by adding organic acids, sodium chloride or some comparable material.

Microscopic counts aid greatly in determining whether or not important types of organisms are failing to develop on the plates prepared. In certain investigations bacteria that were very abundant in the product being studied have been missed entirely because they failed to grow on the medium provided; this likely would not have occurred had the results been checked by microscopic counts.

---

[16] Jr. Bact. 1. P. 481. 1916.

[17] N. Y. (Cornell) Agr. Expt. Sta. Mem. 43. 1921.

[18] U. S. D. A. B. A. I. 28th An. Rpt. P. 225. 1911.

[19] Jr. Bact. 5. P. 565. 1920.

CHAPTER II

MILK FERMENTATIONS

GENERAL CONSIDERATIONS

A milk fermentation may be defined as a process by which a change is produced in milk as a result of the activity of one or more species of micro-organisms. In the condition in which milk normally leaves the udder, it is very satisfactory for the growth of many types of organisms and, accordingly, undergoes a whole series of changes, the processes by which these changes are produced being referred to as fermentations. Many of the fermentations that occur in milk also occur in cream, skim milk, buttermilk and whey.

Normal and abnormal fermentations. The common change occurring in milk is the development of lactic acid with the resulting coagulation of the casein. The process bringing this about is designated the lactic acid fermentation or the souring of milk. Because of the regularity with which this occurs under the usual conditions, it is sometimes called the normal fermentation of milk. The processes causing other changes, such as sweet curdling, the development of ropiness, gases, colors, flavors, odors, etc., constitute the abnormal milk fermentations.

Many milk fermentations may be caused by more than one species and a number of them by a whole series. However, the most convenient basis for a study of the changes occurring in milk as a result of the action of micro-organisms is a division into the different types of changes rather than an attempt to classify all of the causative organisms. The principal reason for this is that the change taking place is the important thing under practical conditions and, frequently, information with reference to a certain fermentation can be given without any knowledge as to the specific organism involved so that valuable time may be saved in correcting the difficulty. If a consideration of the changes occurring in milk were based on a classification of the organisms responsible, the first step in dealing with any abnormal condition would be the isolation and identification of the causative species and this is frequently very time-consuming. An objection to the plan of studying the different fermentations as units is that organisms not at all related are often thrown together because they happen to have one common character, such as the development of gas or ropiness in milk; for example, a discussion of the gassy fermentation involves a consideration of such widely differing organisms as *Torula cremoris* and those of the Escherichia-Aerobacter group.

Mixed fermentations. It frequently happens that two or more fermentations take place in milk at the same time; the production of acid may be accompanied by gas formation or a color change, the development of ropiness may occur along with changes in the flavor and aroma or there may be various other groupings. Often these combinations of fermentations are due to the activity of more than one species but some organisms, in pure culture, produce two or even more changes in milk. The Escherichia-Aerobacter group produces acid, gas and off flavors and aromas in milk and certain cultures also produce ropiness so that it should be considered under at least four fermentations; while this is an extreme example there are many species that produce more than one type of change.

Variations in organisms. Organisms that apparently belong to the same species may differ in a character that is important from the standpoint of milk fermentations. The best illustrations of this are found in connection with ropiness; some cultures of *Lactobacillus bulgaricus* produce a ropy condition while others do not and this same variation occurs with a number of species, including *Lactobacillus casei* and *Streptococcus lactis.* Variations in flavor and aroma production also seem to occur among organisms apparently belonging to the same species.

Associative action. Associative action is the combined action of two, or possibly more, organisms in producing a change which can not be produced by one of the organisms alone. The action of the organisms growing together may often be traced to the influence of the products of one on the rate of growth or the products of the other but, in some instances, no such simple explanation seems to be justified.

The effect of organisms working in combination is particularly important in dairy products and may influence the amount of acid produced, the development of ropiness, color production, volatile acid production, flavor and aroma development, etc. It is probable that associative action is very often involved in the changes occurring in milk and cream on extended holding, especially changes in flavor and aroma. The failure to isolate an organism capable of producing a certain abnormal fermentation in a dairy product is occasionally due to the change being the result of the action of organisms in combination. The species involved when a pronounced change in milk is due to associative action are often present in considerable numbers so that isolations are not difficult.

THE LACTIC ACID FERMENTATION

The process by which lactic acid is produced in milk, cream, etc., through the action of micro-organisms constitutes the lactic acid fermentation. In milk and cream at favorable temperatures enough acid is commonly produced to precipitate the casein and the liquid thus becomes a semi-solid mass. The lactic acid is formed from the lactose and there are many different bacteria that are capable of bringing about this transformation.

Frequency of the fermentation. The lactic acid fermentation occurs regularly in raw milk in all parts of the world, its rate being determined primarily by the temperature at which the milk is held. In rare instances in new dairy districts, the souring of the milk seems to be markedly delayed; this is probably due to the fact that the surroundings are not so thoroughly seeded with lactic acid organisms as in the older dairy districts and the milk is, accordingly, but very lightly infected. Lactic acid is produced through bacterial activity in a variety of materials, such as silage and sauerkraut, in addition to milk and cream.

Historical. Although the presence of living organisms in sour milk was observed very early in the eighteenth century, it was not until 1837[1] that these were suggested as the cause of the souring. The idea of the causal relationship of organisms to this change found only a few advocates until Pasteur,[2] in 1857, definitely showed the existence of lactic acid producing organisms; in 1878, Lister[3] succeeded in isolating one of these forms and thus provided incontrovertible proof. At one time, before the importance of organisms in bringing about various decompositions was recognized, the change from lactose to lactic acid was thought by certain investigators to be due to something in the casein.

[1] Compt. Rend. Acad. Sc. 5. P. 822. 1837.

[2] Compt. Rend. Acad. Sc. 45. P. 915. 1857.

[3] Quar. Jr. Mic. Sc. P. 177. 1878.

Lactic acid. Lactic acid was discovered in sour milk by Scheele, in 1780, but it was not until many years later that its constitution was established as CH_3—CHOH—COOH (alpha-hydroxypropionic acid). It is non-volatile and is miscible with water, alcohol or ether in all proportions. Commercial lactic acid is a colorless, somewhat viscous liquid having a strongly acid taste and containing water. By distilling the aqueous solution under very low pressure (1 mm.), lactic acid can be purified and obtained as a crystalline solid melting at 18° C. (64.4° F.); when heated at ordinary pressure in an attempt to remove the water, it is partially converted into the anhydride before dehydration is complete. Pure lactic acid is odorless.

Transformation of lactose. Although lactose has long been recognized as the source of the lactic acid produced in milk, the reactions representing the transformation have never been definitely proven. They are commonly generalized as follows:

$$C_{12}H_{22}O_{11} + H_2O = 4C_3H_6O_3 \text{ (lactic acid).}$$

The changes taking place in the formation of lactic acid from glucose or galactose are sometimes considered to be the following:

CH_2OH—$(CHOH)_4$—CHO → $2CH_2OH$—CHOH—CHO (glyceric aldehyde) − $2H_2O$ → $2CH_3$—CO—CHO (pyruvic aldehyde) + $2H_2O$ → $2CH_5$—CHOH—COOH (lactic acid).

The transformation of lactose to lactic acid is not quantitative, however, and the fermentation yields other products, the so-called secondary products, which may include various acids, aldehydes and alcohols and, with certain organisms, gases. These secondary products are very important since they largely determine the aroma of the fermented milk and also play a part in determining the flavor. The nature and quantity of the secondary products vary greatly with the different organisms that are capable of producing lactic acid in milk.

Optical isomers of lactic acid. Lactic acid is known in the three isomeric modifications that are to be expected from the asymmetry of the alpha carbon atom. Some organisms produce *d* acid only, others produce *l* only, while still others produce both *d* and *l*; of the organisms that produce both, some form the two in equal amounts, thus giving *i* acid only, while others form the two in unequal amounts and, accordingly, give *i* acid together with *d* or *l*. It seems that the isomeric modification produced is constant for a given organism regardless of the holding temperature, although with organisms producing both *d* and *l* acid the one produced in the smaller amount may be absent under conditions unfavorable for growth. In naturally contaminated milk allowed to sour at room temperature, there is commonly an excess of *d* lactic acid while, if it is allowed to sour at 37° C. (98.6° F.), there is usually an excess of *l* acid. This difference in the predominating form of the acid is due entirely to the variation in the lactic acid organisms favored by the two temperatures.

Acidity of fresh milk. Samples of freshly drawn, normal milk, titrated with phenolphthalein as an indicator, show acidities varying from 0.12 to 0.24 per cent, calculated as lactic acid, which is the usual method of recording acidities in milk and cream under dairy plant conditions.

The *p*H values of over 300 samples of fresh milk, each representing only a portion of the milking, were determined by Van Slyke and Baker[4] and found to range from 6.50 to 7.20, with 80 per cent of the samples under 6.76. The acidity of fresh milk is not due to lactic acid but to acid phosphate,[5] casein, carbon dioxide and probably certain other compounds normally present. Variations in the acidity of fresh milk accompany variations in the composition. Van Slyke and Baker[6] noted that, in general, with a decrease in acidity there is a marked tendency toward a decrease in specific gravity and in the percentages of fat, total solids, solids not fat, casein and lactose but an increase in proteins other than casein and in ash and chlorine. These investigators also found with 20 cows that the *p*H value of milk from different quarters of an animal commonly showed variations, although in most cases they were not large. There is often some fluctuation in the acidity throughout the milking, the fore milk being comparatively low, the middle milk highest and the strippings between the two. This is the variation that would be expected from the composition of the different portions of the milking, if the fact that the fat carries little, if any, acidity is taken into account. The influence of the feed on the acidity of milk is apparently negligible, Turner and Beach[7] noted no effect from silage and Sommer and Hart[8] added up to 120 cc. of concentrated H_2SO_4 to the ration of a normal cow without affecting the acidity of the milk.

Changes in the acidity of milk. When ordinary raw milk is allowed to stand at room temperature the acidity soon begins to increase as a result of the activity of micro-organisms. The acidity at which milk tastes sour varies with different persons and also with the initial acidity, a definite increase in acid being more important than the actual acidity at the time of tasting. Milk having an acidity of 0.3 per cent tastes sour to most people while with many persons an acidity of 0.25 per cent tastes sour. Persons accustomed to judging the flavor of milk and milk derivatives frequently detect an acid flavor in milk with lower acidities, sometimes even under 0.2 per cent, when the milk had a low initial acidity. Schultz, Marx and Beaver[9] reported that an acidity in milk is first detectable by the taste at a *p*H of about 6.0. As the acid continues to increase it eventually causes a precipitation of the casein, the point at which this occurs varying with different samples of milk and usually falling between 0.50 and 0.65 per cent. Van Slyke and Baker[10] found that the casein begins to coagulate when the *p*H value reaches 4.64 to 4.78. On boiling, milk commonly curdles at an acidity varying from 0.30 to 0.45 per cent.

---

[4] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 70. 1919.

[5] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 37. 1914.

[6] See ref. 4.

[7] Conn. (Storrs) Agr. Expt. Sta. 16th An. Rpt. P. 150. 1904.

[8] Jr. Dairy Sc. 4. P. 7. 1921.

With many of the lactic acid producing bacteria the acidity continues to rise after the coagulation of the casein until the accumulation of acid prevents further growth. The maximum amount of acid developed in milk varies widely with different species and even with different cultures of the same species. The lactose, which averages about 5.1 per cent in milk, is very rarely, if ever, the limiting factor in acid production and, under ordinary conditions, there is still considerable lactose remaining when the development of acid has stopped. Van Slyke and Bosworth[11] found that in fresh milk inoculated with two of the common lactic acid organisms, *Streptococcus lactis* and *Aerobacter aerogenes,* and allowed to stand at room temperature for 60 hours, 22 per cent of the lactose disappeared and that 88.5 per cent of the amount used was converted into lactic acid.

The spontaneous souring of milk ordinarily involves chemical changes other than the formation of lactic acid. Van Slyke and Hart[12] have pointed out that the acid produced combines with the bases of some of the inorganic salts and then with the calcium that is in combination with the casein, which results in the formation of a precipitate of free casein. As more acid is produced the free casein unites with it, forming a casein salt of the acid, and this makes up the curd noted in sour milk. Bosworth and Prucha[13] found that the citric acid present disappears during the souring of milk.

---

[9] Jr. Dairy Sc. 4. P. 1. 1921.

[10] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 65. 1918.

[11] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 48. 1916.

[12] N. Y. (Geneva) Agr. Expt. Sta. Bul. 261. 1905.

According to Van Slyke and Baker,[14] lactic acid exists in sour milk largely as lactate but partly as free acid; a portion of the latter is in solution while a smaller part, about 20 per cent in coagulated sour milk, is adsorbed by the casein. In freshly separated skim milk pasteurized at 62° C. (143.6° F.), cooled to 25° C. (77° F.), inoculated with *Streptococcus lactis* and then held at 25° C., free lactic acid appeared in appreciable amounts after about 20 hours, when it equalled 0.1 cc. of n/10 acid in 100 cc. of milk; it increased more rapidly in the next few hours and, after 48 hours, equalled 20 cc. of n/10 acid.

Relationship between titratable acidity and pH. Sharp and McInerney[15] have studied the relationship between titratable acidity and *p*H, using electrometrical *p*H determinations. With samples of fresh milk, some abnormal, the titratable acidity ranged from 0.50 to 0.05 per cent and the *p*H from 6.0 to 7.73. A relation between the titratable acidity and *p*H of fresh milk was found by means of which the *p*H can be determined from the titratable acidity with an average error of 0.06 *p*H, provided the titratable acidity is greater than 0.10 per cent. A different relationship exists between the titratable acidity and *p*H of sour milk as a result of which fresh milk can generally be recognized.

Preservative action of lactic acid. The lactic acid produced in milk prevents the growth of many species of bacteria, especially putrefactive types because of the lack of acid tolerance among these organisms, and thus acts as a preservative. Rapid decomposition of sour milk does not occur until the acidity has been decreased. Under the usual conditions this decrease is brought about by various microorganisms, particularly molds and yeasts, which use a part of the acid and neutralize a part by certain products of their metabolism. In raw milk allowed to stand at ordinary temperatures, the development of acid, a decrease of this acid largely through the growth of molds and yeasts and then a rapid decomposition constitute a fermentation cycle which occurs with great regularity.

However, with sour milk and cream objectionable flavors and aromas, other than those of a putrefactive nature, develop on standing so that the holding period is more or less limited under ordinary conditions. This change is due, in the main, to the activity of micro-organisms but chemical action probably also plays a part in certain instances. The micro-organisms capable of growing in the presence of considerable acid are of various types, with the molds and yeasts of special importance; certain organisms even grow much more rapidly in sour milk than in the sweet product.

---

[13] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 14. 1910.

[14] See ref. 10.

[15] Jr. Biol. Chem. 75. P. 177. 1927.

Types of lactic acid organisms. At the time (1878) that Lister[16] made the first isolation of a lactic acid organism there were still many people who doubted the causative relationship of micro-organisms to fermentations. With the development of a technique satisfactory for the study of bacteria, the action of different organisms in changing a portion of the lactose in milk over to lactic acid was established by various investigators. It is now generally recognized that there are many species of bacteria capable of producing lactic acid in milk and that these show pronounced differences.

The bacteria which are of most importance in the production of lactic acid in milk, cream, etc., fall into three distinct groups as follows: (1) the *Streptococcus lactis* group, (2) the Lactobacillus group, and (3) the Escherichia-Aerobacter group. The *S. lactis* and Lactobacillus groups are commonly referred to as the true lactic acid bacteria since lactic acid is the strikingly prominent product formed by them while, with the Escherichia-Aerobacter group, the fermentation of lactose yields gases and various other compounds in considerable quantities in addition to lactic acid. There are certain organisms that produce lactic acid in milk that are not included in these three groups but most of them are of primary significance because of some other character. Among the more important of these are *Streptococcus liquefaciens,* which has marked proteolytic powers in addition to its ability to attack the carbohydrate of milk, and *Streptococcus paracitrovorus*, which is one of the organisms associated with *S. lactis* in the cultures used in buttermaking and cheesemaking.

THE STREPTOCOCCUS LACTIS GROUP

The organism isolated by Lister[17] (1878) and named *Bacterium lactis* by him apparently belongs to what is now known as the *S. lactis* group. Leichman[18] (1896) proposed the name *Bacterium lactis*

---

[16] See ref. 3.

[17] See ref. 3.

[18] Centbl. f. Bakt. 2 Abt. 2. P. 777. 1896.

acidi for what was undoubtedly the same organism and Kruse[19] (1903) suggested *Streptococcus lacticus* because he considered the organism a Streptococcus. *Bacterium güntheri* has also been employed for this group. The name *S. lactis* is, at present, used since it retains the species name given by Lister and replaces the generic name used by him with one in agreement with the usual systems of nomenclature.

S. lactis refers to a group of organisms, the members of which show variations in the flavor and aroma produced, the rate of acid development, the optimum growth temperature, the reducing power, the arrangement of the cells, etc. These differences are reasonably constant, although probably somewhat influenced by various factors, and are of considerable significance in dairying because of the great frequency with which these organisms develop in dairy products. On the basis of variations commonly encountered, Hammer and Baker[20] have proposed a number of types of *S. lactis* and there are undoubtedly others that are also of importance; the types proposed are as follows:

Typical *S. lactis* which shows no unusual characters.
S. lactis var. maltigenes—malty *S. lactis*—which produces a malt-like or caramel flavor and aroma in milk.
S. lactis var. hollandicus—ropy *S. lactis*—which produces a ropiness in milk.
S. lactis var. anoxyphilus—slow reducing *S. lactis*—which reduces litmus and Janus green in milk comparatively slowly.
S. lactis var. tardus—slow coagulating *S. lactis*—which coagulates milk slowly.

A heat resistant lactic acid producing Streptococcus is closely related to the above types but shows differences enough so that a distinct species name, *Streptococcus thermophilus,* is used for it.

Morphology. The various names that have been proposed for the organisms now designated as *S. lactis* indicate that there has been some difference of opinion as to the morphology. At present, however, they are generally considered as practically spherical forms that may be slightly longer than broad, especially just before division. The size of the cells in different cultures varies a great deal and may be influenced by the age of the culture, the material in or on which they are grown, the temperature of incubation, etc.; the diameter usually falls between 0.4 and 1.0 micron. When grown in milk the organisms are ordinarily arranged in pairs or short chains, although cultures showing long chains are occasionally encountered; rarely, the pairs of organisms are grouped into clumps. In liquid media other than milk chains may be formed, while on solid media they are unusual. The organisms are gram positive and stain readily with the ordinary stains. They are non-motile and do not form spores. Capsules are often present although usually found on only a portion of the cells in any preparation.

---

[19] Centbl. f. Bakt. 1 Abt. 34. P. 737. 1903.

[20] Ia. Agr. Expt. Sta. Res. Bul. 99. 1926.

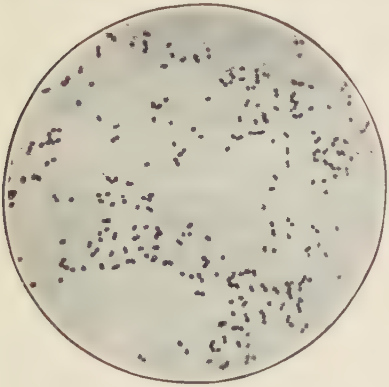


Fig. 1.—*Streptococcus lactis* in milk.

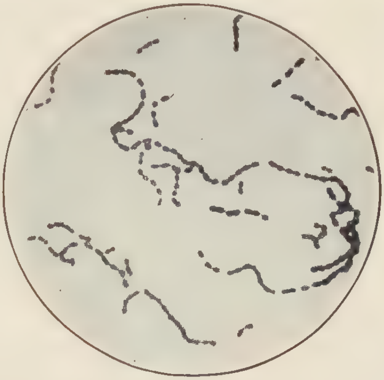


Fig. 2.—*Streptococcus lactis* showing long chains. From a butter culture. Iowa Agr. Expt. Sta. Res. Bul. 22.

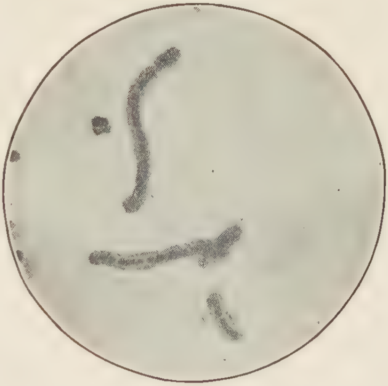


Fig. 3.—*Streptococcus lactis* showing capsules.

Cultural characteristics. The *S. lactis* organisms grow best in milk and milk derivatives. They develop poorly on agar made with beef extract but fairly well on whey and beef infusion agars. The growth in bouillon is meager, unless some material that the organisms are capable of fermenting is added. The most characteristic of the cultural reactions is that in litmus milk. Ordinarily, the first observable change is a reduction of the litmus which usually begins at the bottom and progresses toward the top; often reduction of the litmus is practically complete before any coagulation is evident, although this is not the case with *S. lactis* var. *anoxyphilus*. Coagulation also commonly begins at the bottom and, when this is complete, it is usual to find the milk white, except for a slight pink band at the surface. As the culture ages this pink band gradually increases in width until the whole tube has become pink. There is no digestion or gas formation.

The colonies on agar or gelatin are small, white, smooth edged and non-viscous. Commonly the surface colonies are round and the sub-surface colonies ellipsoid.

Bio-chemical characteristics. *S. lactis* cultures ordinarily ferment glucose, galactose, fructose, lactose and maltose, while some of them ferment sucrose; other common test materials may or may not be fermented. Lactic acid is regularly produced from the lactose in milk and, at favorable temperatures, the rate is very rapid; other acids, mainly acetic and propionic, are also formed. The maximum acidity developed in milk by most cultures is between 0.7 and 1.0 per cent, although occasionally an acidity up to 1.25 per cent is encountered. The percentage of the theoretical yield of lactic acid (according to the reaction $C_{12}H_{22}O_{11} + H_2O = 4C_3H_6O_3$) that is produced by *S. lactis* varies from 88 to 98 per cent. The lactic acid formed is of the *d* type, regardless of the temperature at which the fermentation occurs. By chemical means a small amount of proteolysis can be detected in milk in which certain cultures of *S. lactis* have grown; this proteolysis is increased if $CaCO_3$ is added and the milk then frequently shaken during the fermentation to prevent the rapid accumulation of acid. Gelatin is not liquefied.

S. lactis grows well over a wide temperature range, with different cultures showing different temperature extremes. Most cultures exhibit their maximum growth, as measured by the rate of acid development, between 30° and 40° C. (86° and 104° F.), although a few cultures do not grow at 37° C. (98.6° F.). Below 10° C. (50° F.) growth is very slow, if it occurs at all. In general, the maximum acidity produced is somewhat less at the higher than at the lower temperatures.

S. lactis is facultative; ordinarily, acid production is somewhat more rapid with a restricted than with an abundant air supply.

Identification of S. lactis. The identification of *S. lactis* can be made satisfactorily on the basis of the cultural and morphologic characters shown in litmus milk. Any organism coagulating litmus milk rapidly or fairly rapidly, with reduction of the litmus but without digestion or the formation of gas, and which appears in stains from milk as a gram positive coccus arranged in pairs or chains, the pairs rarely being grouped into clumps, is to be considered as belonging to this group. For many purposes the variety of *S. lactis* to which the organism belongs should be determined.

Fermenting capacity of S. lactis cells. The lactic acid produced, per individual cell per hour, in milk by *S. lactis* was found by Baker, Brew and Conn,[21] using the direct microscopic count, to lie between 5×10^{-10} and 10×10^{-10} mgs. for a vigorous culture; the rate tended to decrease as the curdling point was approached. Considerable variation occurred in the rate of acid production with different cultures. The ratio of the total acid developed to the number of organisms present was fairly constant. There was a tendency for the acid contained, whether measured as titratable acidity or as *p*H, to increase geometrically until coagulation.

It seems to have been common at one time to consider the growth and acid production of *S. lactis* as more or less independent. The recent evidence indicates, however, that there is acid development during the early growth period but that it is difficult to detect because it is so small in amount. No relationship exists between the acidity and the number of cells of *S. lactis* in milk in which this organism is growing because, after the acidity has reached a certain point, the cells begin to die out, due to their inability to tolerate the acid, while the acid production may still slowly continue. Cultures of *S. lactis* in milk die rather quickly and this is especially true at higher temperatures.

Enzyme action in acid production. The action of an enzyme in the production of acid has been shown by Hastings, Evans and Hart,[22] who found that the acidity increased in milk which was heated to destroy the inherent milk enzymes, inoculated with *S. lactis* and then, after the organism had grown for a while, preserved with three per cent toluol. Inoculation experiments showed growth could not have persisted after the addition of the toluol and that, within a short period, the lactic acid organisms were destroyed, while microscopic examinations showed a disintegration of the cells after some time. The following data, selected from those presented by these investigators, illustrate the acid increase:

---

[21] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 74. 1919.

[22] Wis. Agr. Expt. Sta. Res. Bul. 25. 1912.

Increase of Acidity in Milk Preserved with 3 Per Cent Toluol After the Growth of *S. lactis*

	Initial Acidity	Acidity After						
		1 Day	13 Days	28 Days	59 Days	90 Days	121 Days	150 Days
Experiment 1:								
Per cent acidity......	0.349	0.427	0.423	0.460	0.428	0.492	0.540	0.543
Increase in acidity, per cent..............	......	0.078	0.074	0.111	0.079	0.143	0.191	0.194
Experiment 2:								
Per cent acidity......	0.456	0.494	0.519	0.552	0.528	0.599	0.680	0.708
Increase in acidity, per cent..............	......	0.038	0.063	0.096	0.072	0.143	0.224	0.252

Acids, other than lactic, formed by S. lactis. The distinct acid odor present in cultures of *S. lactis* suggests that acids other than lactic have been formed and determinations on *S. lactis* cultures show a definite but rather small amount of volatile acid. Evans[23] reported that cultures of *S. lactis* produced a fairly constant quantity of acetic acid in milk, equivalent to about 0.12 of a gram for one liter of milk. Hammer and Sherwood,[24] using the methods of determining the barium and Duclaux values, found that the volatile acidity produced by *S. lactis* was made up of acetic acid with considerable amounts of propionic.

Associative action in the production of acid by S. lactis. Various organisms common in dairy products have an effect on the amount of acid produced in milk by *S. lactis*. Marshall and Farrand[25] found that 24 of 42 organisms isolated from milk accelerated the growth, 11 had no influence while 7 retarded it. The acceleration occurred with various types of organisms and at different stages in the development of *S. lactis;* it was apparently due to products formed by the associated organism and these were sometimes stable to heat and sometimes not. In mixtures of *S. lactis* and other organisms, the latter sometimes disappeared and sometimes persisted and caused abnormal lactic acid fermentations. In certain instances the relative numbers of *S. lactis* and the other organism had a decided influence

[23] Jr. Agr. Res. 13. P. 235. 1918.

[24] Ia. Agr. Expt. Sta. Res. Bul. 80. 1923.

[25] Mich. Agr. Expt. Sta. Spec. Bul. 42. 1908.

on the acceleration of the growth of *S. lactis.* From investigations reported,[26] it appears that when certain yeasts are grown in combination with *S. lactis* in milk the length of time this organism retains its vitality is greatly increased.

Importance of S. lactis. *S. lactis* is the organism mainly responsible for the souring of milk and cream under practical conditions. In this way it causes great economic losses since sour milk and cream can not be used for many purposes for which the sweet products can be employed and are, accordingly, less valuable. One of the organisms belonging to the *S. lactis* group, *S. lactis var. maltigenes,* produces an objectionable flavor and aroma in milk and cream. Because this condition is not removed by pasteurization, it may be present in milk, cream, butter, etc., in which there are none of the responsible organisms in a living condition.

S. lactis plays a necessary part in the preparation of certain dairy products and is, therefore, beneficial as well as harmful. It is important in the ripening of cream for buttermaking, in the manufacture and ripening of many types of cheese and in the making of various fermented milks, its principal function in all these cases being the development of acid.

Distribution and sources of S. lactis. At the time milk leaves the stable it practically always contains *S. lactis* but the percentage of the total flora made up of this organism is ordinarily small. It does not come from the interior of the udder and, in experiments involving the inoculation of *S. lactis* into the udder through the teat canal, the organism quickly dies out so it seems that the interior of the udder is unsuited to its development. Perhaps the most common source of *S. lactis* under practical farm conditions is the utensils. Esten[27] found it in the feces of 11 out of 25 cows and, with 17 of 21 cows, it was secured from the mouth; the mangers and any materials within reach of a cow's mouth were well seeded with it. McGuire[28] found the organism to be a normal constituent of cow feces. *S. lactis* can commonly be secured from the coat of a cow, its presence there presumably being due to the animal licking herself and to contamination with fecal material. It is also present in silage and on various grains.

Isolation of S. lactis. The isolation of *S. lactis* is easily accomplished by allowing raw milk or cream to sour at ordinary temperatures, plating out on whey or beef infusion agar, incubating the plates at room temperature and then picking colonies into litmus milk. In recently soured milk *S. lactis* ordinarily makes up from 90 to 99 per cent of the total flora so that most of the colonies picked will be *S. lactis,* even when no attempt at a selection is made. The colonies of *S. lactis* can easily be distinguished from those of many, but not all, of the organisms common in milk. When an attempt is made to isolate *S. lactis* from fresh sweet milk by direct plating, difficulty is frequently encountered because of the small numbers in which the organisms are present and the lack of anything characteristic about the colonies.

---

[26] Mich. Agr. Expt. Sta. Tech. Bul. 15. 1912.

[27] Conn. (Storrs) Agr. Expt. Sta. Bul. 59. 1909.

[28] Johns Hopkins Hosp. Bul. 26. P. 386. 1915.

Pathogenicity of S. lactis. Heineman [29] concluded from his experimental data that *S. lactis* is closely related to *Streptococcus pyogenes,* not only morphologically and culturally but also in pathogenic properties. He found that by repeated passage of *S. lactis* through rabbits its virulence was gradually increased and that, after five or more passages in subcutaneous injections, it became fatal to rabbits in doses of two cc. of 24 hour broth cultures. The lesions produced by *S. lactis* in rabbits were found to be of the same kind and extent as those produced by *S. pyogenes.*

In unreported experiments carried out at the Iowa Agricultural Experiment Station by H. A. Bendixen, various milk cultures of *S. lactis* inoculated into either rabbits or guinea pigs were not harmful. Cultures injected into rabbits, intravenously, could be recovered from the spleen and liver for a number of hours after inoculation but repeated passage through rabbits in this way failed to yield cultures which were capable of causing death in the rabbits into which they were inoculated.

THE LACTOBACILLUS GROUP

The Lactobacillus or rod shaped lactic acid group includes the true lactic acid bacteria that are definite rods. In litmus milk there is commonly reduction, without gas formation or digestion, so that a smooth curd, similar to that secured with *S. lactis,* is ordinarily produced; a gas forming type, *Lactobacillus acidophil-aerogenes,* has been described by Torrey and Rahe [30] and isolated by various investigators. Some of the Lactobacillus cultures encountered produce acid in milk very slowly and in this respect resemble the slow acid producing

---

[29] Jr. Inf. Dis. 4. P. 87. 1907.

[30] Jr. Inf. Dis. 17. P. 437. 1915.

S. lactis cultures; the acid production of such cultures can often be increased by a series of transfers through milk. The lactobacilli grow poorly on agar of various compositions so that they are not ordinarily noted in plates poured in the usual way, even when they are present in large numbers in the material plated.

Presence of lactobacilli in dairy products. The lactobacilli appear to be very regularly present in dairy products. Following investigations of these organisms in Europe, Hastings and Hammer[31] reported their wide distribution in milk, butter and cheese in the United States and Heineman and Hefferan[32] found them normally present in feces of various animals, in a variety of sour and aromatic foods, such as dill pickles, olives and sauerkraut, in cattle feed, in normal saliva and gastric juice, in various fermented milks, in market milk and in soil, both manured and unmanured. The lactobacilli play but little part in the beginning acid development in milk and cream because of the rapid growth of other acid producing types, especially *S. lactis*, but may become active later when these other organisms have produced their maximum acidities. In certain of the dairy sections producing sour cream for buttermaking, the cream occasionally shows acidities over 2 per cent during the warm months and this is largely due to the action of the lactobacilli contained. Whey in vats and as returned to the farms was found by Dotterrer and Breed[33] to contain large numbers of these organisms. They are also commonly present in ripened cheeses, often in large numbers.

The lactobacilli are well adapted to growth in dairy products generally. They are greatly favored by the lactose present and their acid tolerance permits their development under conditions which inhibit many of the bacteria.

Importance of the lactobacilli in dairy products. The development of a high acidity in cream intended for churning is very undesirable because of the influence on the flavor of the butter. This effect may be decreased by partial neutralization but it can not be entirely controlled. In the ripening of certain cheeses, however, the lactobacilli play an important role since they are one of the factors involved in the protein decomposition. The lactobacilli are also desirable in certain of the fermented milks.

Sources of the lactobacilli in milk. Experiments carried out at the Iowa Agricultural Experiment Station indicate that milk does not contain lactobacilli when it leaves the udder but that they are present by the time the milking is complete. These organisms are regularly found in the stable surroundings. Grains and various other feeds contain them. Hunter and Bushnell,[34] Sherman,[35] and Heineman and Hixson [36] have shown that enormous numbers are present in silage; Sherman reported that they are constantly found in quite large numbers on corn fodder so that corn silage is always amply seeded. These organisms are also present in cow manure. Their presence in various feeds partly accounts for this but a multiplication in the intestinal tract may also be a factor. Orcutt [37] found that these organisms were always present throughout the small intestine of young calves and were the predominating type in the fourth stomach.

---

[31] Wis. Agr. Expt. Sta. Res. Bul. 6. 1909.

[32] Jr. Inf. Dis. 6. P. 304. 1909.

[33] N. Y. (Geneva) Agr. Expt. Sta. Bul. 412. 1915.

Isolation of lactobacilli from dairy products. Lactobacilli are not readily isolated because of the difficulty with which they grow on media adaptable to plating. In isolating from milk a bottle should be filled, stoppered with a cork and then held at a favorable temperature, such as 37° C. (98.6° F.), so that the desired organisms will increase in numbers. *S. lactis* and various other species will multiply rapidly at first but, when these are restrained by the acid developed, the lactobacilli will multiply. Microscopic examinations make it possible to follow the fermentation. When the lactobacilli are present in large numbers the milk can be plated on whey or on beef infusion agar, the plates then incubated at 37° C. and colonies picked into litmus milk. Growth on agar is frequently very poor and colonies may not develop even when large numbers are present in the milk. In some instances shake cultures in tubes have yielded a better colony development than plates, due presumably to the oxygen requirements of the organisms. It is sometimes an advantage to transfer a loop of the fermented milk from the first bottle to a bottle of sterile milk and allow this to develop acid, with the idea that the desired organisms will so dominate the fermentation that little difficulty will be experienced from other species. It is necessary to have the bottles practically full and stoppered tightly so that there will be little opportunity for the growth of molds. Lactose fermenting yeasts sometimes cause considerable trouble by blowing the stoppers and forcing out some of the milk.

The acid tolerance of the lactobacilli makes it possible to check other types by means of added acid. One of the procedures used is to inoculate the material from which the isolation is to be made into bouillon to which a fermentable material, such as glucose, and enough acetic acid to give approximately an n/10 solution have been added. Growth can be followed microscopically and, if the lactobacilli develop satisfactorily, plates can be poured. A series of transfers through the acid bouillon may yield essentially a pure culture of lactobacilli.

---

[34] Kan. Agr. Expt. Sta. Tech. Bul. 2. 1916.

[35] Jr. Bact. 1. P. 445. 1916.

[36] Jr. Bact. 6. P. 45. 1921.

[37] Jr. Bact. 11. P. 115. 1926.

Species of the lactobacilli important in dairying. There are three species of the lactobacilli that are especially important in the field of dairying. These are *L. casei, L. acidophilus* and *L. bulgaricus.*

The organism now known as *L. casei* was apparently first studied by von Freudenreich [38 and 39] in investigations on the ripening of Swiss cheese and its chief importance still appears to be in connection with cheese ripening. It is quite regularly present in milk in the United States and at least certain other countries; for example, Steuart [40] found lactobacilli invariably present in milk at Cardiff, Wales, and it seems probable that they should be classed as *L. casei.* Sherman and Stark [41] noted that *L. casei* is generally present in milk in considerably larger numbers than either *L. acidophilus* or *L. bulgaricus.* With grade A milk, only 12 per cent of the samples contained *L. acidophilus* or *L. bulgaricus* in numbers as great as one per cc. while 71 per cent contained *L. casei* in numbers that large; with ordinary milk, only 2.3 per cent of the samples contained *L. acidophilus* or *L. bulgaricus* in numbers exceeding ten per cc. while 94 per cent contained *L. casei* in excess of ten per cc.

L. acidophilus was first isolated from the fecal material of infants by Moro [42] and is now recognized as being more or less regularly present in the normal intestinal tract of man and other warm-blooded animals. Cultures of the organism are used in attempts to control the intestinal flora. It seems to establish itself better than *L. bulgaricus,* due probably to its ability to grow satisfactorily at the low surface tension prevailing in the lower intestine as a result of the presence of bile salts. The consumption of lactose appears to favor the growth of *L. acidophilus* in the intestinal tract.

L. bulgaricus was originally isolated by Grigoroff [43] from fer-

---

[38] Landw. Jahrb. Schweiz. 5. P. 16. 1891.

[39] Centbl. f. Bakt. 2 Abt. 5. P. 241. 1899.

[40] Jr. Dairy Sc. 3. P. 52. 1920.

[41] Jr. Bact. 13. P. 60. 1927.

[42] Jahrb. f. Kinderh. 52. P. 38. 1900.

[43] Rev. Med. d. l. Suisse Romande. 25. P. 714. 1905.

mented milk and called *Bacillus A*. It received considerable attention as a result of the recommendation of Metchnikoff that it be used to control intestinal putrefaction which this investigator believed was an important factor in limiting the length of life in man. The suggestion was based on the theory that, since putrefactive organisms are sensitive to acid, their development in the intestinal tract can be controlled by organisms producing considerable amounts of acid. *L. bulgaricus* was selected by Metchnikoff because it produces a comparatively high acidity in milk and also because of the presence of this organism in the sour milk consumed in large quantities by certain races noted for their long life. Extensive trials have shown, however, that *L. bulgaricus* does not establish itself in the intestinal tract even when consumed in large numbers. At one time this was thought to be due to a lack of a fermentable carbohydrate in the lower intestinal tract and attempts were made to find an organism which would supply suitable materials through the breaking down of the complex carbohydrates; these were unsuccessful because the decomposition of the carbohydrates was carried to comparatively simple bodies that could not be used as a source of acid. It now appears that *L. bulgaricus* is an organism not adapted to conditions in the intestinal tract.

General description of L. casei, L. acidophilus and L. bulgaricus. *Morphology. L. casei, L. acidophilus* and *L. bulgaricus* are definite rods that vary a great deal in size, with *L. bulgaricus* tending to be larger than the others. The cells may occur singly or in chains; some of the *L. bulgaricus* cultures show unusually long chains. The organisms stain readily and are gram positive; negative cells are sometimes noted, especially with *L. bulgaricus.* Occasionally, stained preparations show distinct granules. Spore formation does not occur and the organisms are non-motile.

Cultural characters. Growth appears to be better in milk than in any other material and is generally poor on agar media; whey and beef infusion agars are more satisfactory than beef extract agar. In litmus milk there is ordinarily coagulation and reduction of the litmus, except for a pink band at the top. Gas is not produced. Growth is definite, but slight, in bouillons containing various fermentable materials. Colonies are small and somewhat variable in type in that the edge may be either smooth or filamentous; some *L. bulgaricus* cultures give a very filamentous edge.

Bio-chemical features. Acid is produced from a number of materials but there is no great regularity in those fermented. In milk, acid develops readily at favorable temperatures but the final acidity varies a great deal, with some cultures giving only a slight curd in milk while others produce over 4 per cent acid; *L. bulgaricus* seems to yield especially large amounts. The isomeric form of lactic acid produced is variable and the results obtained by different investigators for any one of the species are not constant. In general, it appears that *L. bulgaricus* tends toward the *i* form, *L. acidophilus* toward the *d* form while *L. casei* is quite variable. The percentage of the theoretical yield of lactic acid is high, up to approximately 98 to 99 per cent, and in accordance with this the cultures are lacking in aroma. A proteolysis of the casein can usually be detected by chemical examination although not suggested by the appearance of the milk cultures.

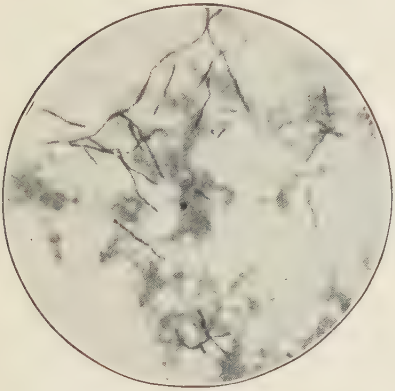


Fig. 4.—*Lactobacillus acidophilus* showing chains.

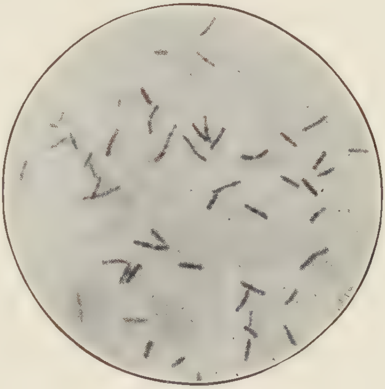


Fig. 5.—*Lactobacillus bulgaricus* showing granules.

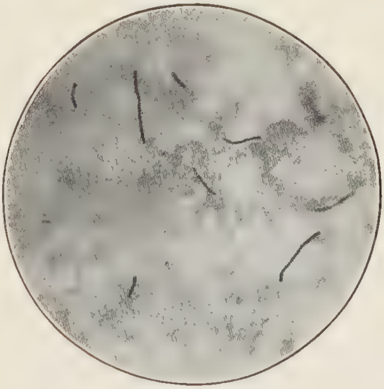


Fig. 6.—Lactobacilli in old, very acid cream.

A comparatively high temperature, such as 32.2° to 37.8° C. (90° to 100° F.), greatly favors growth. The organisms are facultative.

Differentiation of L. casei, L. acidophilus and L. bulgaricus. The close relationship of *L. casei, L. acidophilus* and *L. bulgaricus* is soon evident when these organisms are compared. There appears to be no clearly differentiating characters and the differences that occur are quantitative rather than qualitative. Kulp and Rettger [44] suggest that, although the differences between *L. acidophilus* and *L. bulgaricus* constitute evidence enough for classifying them as separate species, the two types be placed in one species, with *L. acidophilus* the central type and *L. bulgaricus* the variant. These investigators found that the outstanding difference between these organisms was in the habitats, *L. acidophilus* being a normal intestinal form while *L. bulgaricus* is a typical milk type.

Albus and Holm [45] noted that when the surface tension of bouillon was depressed with sodium ricinoleate *L. bulgaricus* was inhibited below 40 dynes while *L. acidophilus* grew down to as low as 36 dynes. This same general relationship occurs in the results secured by various investigators, although occasionally a culture that is believed to be of a certain type does not react to surface tension depressants as would be expected from the name; for example, Kopeloff and Beerman [46] studied nine strains of *L. acidophilus* and six of *L. bulgaricus* and found that, with sodium ricinoleate as the depressant, all of the strains of so-called *L. acidophilus* except one were able to grow at a surface tension of three or more dynes below *L. bulgaricus.*

Sherman and Stark [47] used the growth temperatures in differentiating the three lactobacilli important in dairying. They considered that *L. acidophilus* and *L. bulgaricus* will grow at 45° C. (113° F.) while *L. casei* will not and also that *L. casei* will grow at 15° C. (59° F.) while the other types will not.

Hyde and Hammer [48] studied 12 cultures of lactobacilli, seven of which they isolated from sources that would be expected to yield *L. acidophilus,* while five were secured from commercial or research laboratories. The lactic acid produced varied from practically pure active to practically pure inactive, the active acid being *d* rotary in all cases. There was a tendency toward a correlation of certain characters with the production of lactic acid that was largely or entirely inactive; these included growth at 45° C. (113° F.) but not at

---

[44] Jr. Bact. 9. P. 357. 1924.

[45] Jr. Bact. 12. P. 13. 1926.

[46] Jr. Bact. 13. P. 7. 1927.

[47] See ref. 41.

[48] Ia. State Col. Jr. Sc. 1. P. 419. 1927.

15° to 20° C. (59° to 68° F.), failure to grow at a reduced surface tension and a comparatively large size. The seven isolated cultures and one from a commercial laboratory all produced largely or entirely *d* lactic acid, all grew at 15° to 20° C. but not at 45° C. and six of the eight grew at a reduced surface tension; they should undoubtedly be classed as *L. acidophilus.*

The findings in the various investigations suggest that the differentiation of the lactobacilli that are important from the standpoint of dairy products is very difficult and it appears that the source should be taken into account in all cases.

The Escherichia-Aerobacter Group

The Escherichia-Aerobacter or colon-aerogenes group consists of the gram negative, non-spore forming organisms which produce acid and gas in milk and which are capable of growing aerobically. The rate and extent of acid production vary a great deal, some cultures causing coagulation fairly rapidly while others fail to coagulate even after long holding periods. Gas production is ordinarily conspicuous in young cultures. When coagulation occurs the curd is not especially gassy because the gas largely escapes before the precipitation of the casein takes place. The organisms do not proteolyze the casein in milk, although there is sometimes a collection of whey at the top of a culture that suggests this. Growth on the usual agar plates appears quickly and the mature colonies are large.

Division of the Escherichia-Aerobacter group. There are two main types of organisms included in the Escherichia-Aerobacter group, (1) those belonging to the genus Escherichia, of which *Escherichia coli* is the type species and (2) those belonging to the genus Aerobacter of which *Aerobacter aerogenes* is the type species. Each of these genera includes a number of species. The generic diagnoses for the two types, as given by Weldin,[49] are as follows:

"Genus Escherichia. Motile or non-motile. Gram negative, non-spore-forming rods, fermenting glucose and lactose with both acid and gas. Do not produce acetyl-methyl-carbinol from glucose (Voges-Proskauer negative); reverse the reaction in 0.5 per cent glucose-phosphate-peptone solution very slowly or not at all (methyl red reaction positive); generally not able to utilize uric acid as the sole source of nitrogen. Gelatin not liquefied. Pathogenicity usually slight."

---

[49] Ia. State Col. Jr. Sc. 1. P. 121. 1927.

"Genus Aerobacter. Motile or non-motile. Gram negative, non-spore-forming rods, fermenting both glucose and lactose with both acid and gas. Produce acetyl-methyl-carbinol (Voges-Proskauer reaction positive); reverse the reaction of 0.5 per cent glucose-phosphate-peptone solution relatively rapidly; generally able to utilize uric acid as an available source of nitrogen. Pathogenicity usually slight or absent."

The Escherichia type includes primarily the organisms coming from the intestinal tract of man and animals while the Aerobacter type includes primarily those coming from soil and grains. Rogers, Clark and Evans[50] have called attention to the scarcity of the Aerobacter type in bovine feces. Later these same investigators[51] pointed out that the Escherichia-Aerobacter organisms from grains do not agree with the characteristic flora of bovine feces.

Presence of the Escherichia-Aerobacter organisms in milk. Organisms of the Escherichia-Aerobacter group are practically always present in milk at the time it leaves the stable, even when the most careful methods of production are used. Ayers and Clemmer[52] found that milk produced under the worst conditions normally encountered rarely contained over 2,000 of these organisms per cc. Finkelstein[53] reported that, when care was used in the production of milk, the number of Escherichia-Aerobacter organisms was ordinarily small, averaging less than 100 per cc., while when indifferent methods were used the number averaged 588 per cc.

Growth of the organisms in milk. The Escherichia-Aerobacter organisms grow rapidly in milk when the temperature is suitable and multiplication may be the cause of excessive numbers. Ayers and Clemmer[54] reported that little or no growth occurs below 10° C. (50° F.). Finkelstein[55] also found that this temperature or lower checked the growth of the group in raw milk and that a temperature of 15.6° C. (60° F.) or higher resulted in a rapid increase. Hunter[56] investigated the effect of temperature on the Escherichia-Aerobacter organisms in milk by taking 59 samples from an average dairy and making counts at once and after holding one portion at 15.6° C. and the other at 21.1° C. (70° F.) for 24 hours. The fresh milk averaged 52 Escherichia-Aerobacter per cc. with variations from 1 to 1,000, that held at 15.6° C. averaged 964 per cc. with variations from 0 to

---

[50] Jr. Inf. Dis. 15. P. 100. 1914.

[51] Jr. Inf. Dis. 17. P. 137. 1915.

[52] U. S. D. A. Bul. 739. 1918.

[53] Jr. Dairy Sc. 2. P. 460. 1919.

[54] See ref. 52.

[55] See ref. 53.

[56] Jr. Dairy Sc. 2. P. 108. 1919.

10,000, while that held at 21.1° C. averaged 60,762,000 per cc. with variations from 1,000 to 1,000,000,000.

There is ample evidence that the different species belonging to the group vary in the effect of temperature on their activity. Certain cultures of the Aerobacter type may fail to grow at 37° C. (98.6° F.).

Importance in dairy products. The Escherichia-Aerobacter organisms are always undesirable in dairy products. In milk and cream, in addition to forming acid and gas, they produce objectionable flavors and aromas of a variety of types; when these products are used in the manufacture of cheese or butter the off flavors are commonly carried over. Although the organisms of this group are not such pronounced acid formers as *S. lactis* they may play a part in the natural souring of milk and cream. Heinemann[57] found them in large numbers in the initial stages of souring, while *S. lactis* dominated the field in the later stages. The Aerobacter type can grow in milk at a lower temperature than the Escherichia type so that it is of more importance from the standpoint of development in milk under ordinary conditions.

Sources of the organisms in milk. Organisms of the Escherichia-Aerobacter group have occasionally been found in milk at the time it leaves the udder but this is an unusual condition. They commonly get into milk through the general contamination with manure, soil, feeds of various types, etc. Hunter and Bushnell[58] found them in the various kinds of silage studied. Ayers and Clemmer[59] noted that they can also come from unsterile utensils. In one series of trials in which utensils were allowed to contain milk drainings for 24 hours during extremely hot weather and then washed carelessly in lukewarm water, the Escherichia-Aerobacter count of the fresh milk averaged about 1,000,000 per cc.; an attempt to duplicate these results was unsuccessful.

The data of various investigators show that milk commonly contains both Escherichia and Aerobacter species. Levine[60] summarized data from various sources and found that, of 1,382 cultures isolated from milk, 43.1 per cent belonged to the Aerobacter type. Hunter[61] reported that, of 590 cultures secured from milk, 66.2 per cent belonged to the Escherichia type while, of 169 cultures from bovine feces, 95.2 per cent also belonged there.

Important bio-chemical features. The bio-chemical features of the Escherichia-Aerobacter organisms are important because of the extended growth of these forms in dairy products under certain conditions. Gas and lactic acid are readily produced from lactose, the lactic acid being of the *l* type. The per cent of the theoretical yield of lactic acid is very low and considerable quantities of gas and other products, including especially various volatile acids, are formed.

---

[57] Jr. Inf. Dis. 3. P. 173. 1906.

[58] See ref. 34.

[59] See ref. 52.

[60] Ia. Eng. Expt. Sta. Bul. 62. 1921.

[61] See ref. 56.

Isolation of the Escherichia-Aerobacter organisms from dairy products. Organisms of the Escherichia-Aerobacter group can be isolated directly from dairy products by plating, either on standard agar or some differentiating medium, or an enrichment procedure, using bouillon of various types or lactose bile, may precede the plating. The detection of the organisms in dairy products is often based on the development of gas in some medium. Kessler and Swenarton[62] recently found that gentian violet lactose peptone bile was the most satisfactory, for this purpose, of several media tried and that the formation of gas in this medium, when inoculated with milk or diluted milk, is a positive indication of the presence of the organisms which, for practical purposes, needs no confirmation.

Species of the Escherichia-Aerobacter group important in dairy products. In most of the work done on the Escherichia-Aerobacter organisms, from the standpoint of dairy products, no attempt has been made to carry the identification to a species basis and they have simply been divided into the Escherichia and Aerobacter types. Accordingly, there is little information available on the species that are commonly responsible for abnormal conditions in dairy products.

THE ROPY OR SLIMY FERMENTATION

In the ropy or slimy fermentation of milk, cream, etc., the growth of micro-organisms results in such a change in consistency that these materials can be drawn out into threads or masses. There is a great variation in the extent of the ropiness; sometimes the change is so slight that it can be detected only by a very careful examination while, in other cases, the material can be drawn out into threads a yard or more in length and as fine as the threads of a spider web. In certain instances, particularly with some of the acid producing organisms, the ropy material takes on a tough, doughy consistency and a small container filled with it can often be inverted without spilling. If the ropiness is at all pronounced it is readily evident when the material is poured or when some object, such as a fork or spoon, is drawn through it. In general, the ropiness present in sweet milk or cream disappears as acid develops while that accompanying a definite acid production frequently increases with an increase in acid and then later decreases. Vigorous agitation markedly decreases the ropiness. Normal cream with a high content of fat occasionally shows a consistency which suggests a slightly ropy condition to anyone unfamiliar with this product.

---

[62] Jr. Bact. 14. P. 47. 1927.

Comparison of ropy and "stringy" milk. Milk which has undergone a ropy fermentation should be differentiated from the "stringy" or "gargety" milk coming from certain abnormal udders; with the latter the unusual condition is due to the presence of masses of fibrin and leucocytes thrown out by the udder tissues in response to an infection, while with the ropy milk the consistency is due entirely to the growth of bacteria in what was originally a normal product. "Stringy" milk shows the abnormal condition at the time it is drawn and, when not evident from the appearance of the milk itself, is commonly recognized by the material collecting in the strainer cloth or clarifier bowl, while ropiness is not generally present until the milk has been held for at least 12 hours.

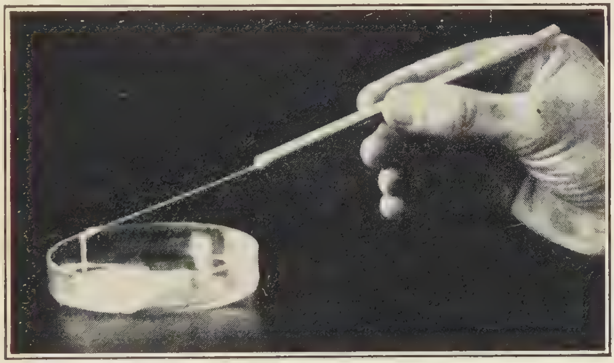


FIG. 7.—Ropy cream. Causative organism *Bact. viscosum.*

Importance of ropiness. Ropiness is one of the common abnormal fermentations of milk and its liquid derivatives. Undoubtedly it is most frequently met with and is the most serious in market milk, where it is the cause of a great deal of annoyance to dealers. Milk may be delivered in a normal condition and then show ropiness after being held by the consumer. This often occurs when the holding temperature is quite satisfactory, due to the fact that some of the organisms producing ropy milk grow unusually well at fairly low temperatures and determine the type of change taking place there while, at higher temperatures, they are overgrown by the lactic acid organisms and the milk sours. Very often only the cream is ropy and the milk beneath is normal; this is because of the oxygen requirements of certain of the causative organisms. Milk consumers frequently assume that ropiness is due to a diseased condition in the producing animals and, unless the dealer quickly brings the trouble under control, a big loss in patronage is likely to follow an outbreak.

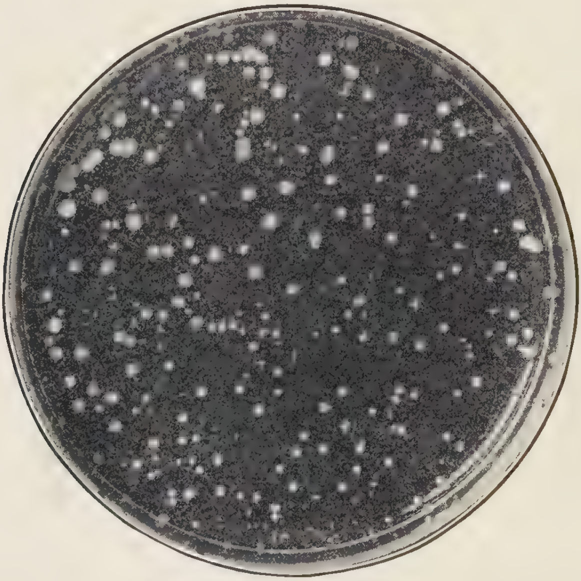


Fig. 8.—Plate poured from ropy cream. Causative organism of the Escherichia-Aerobacter type.

The organisms causing ropiness seem to be entirely harmless to persons using milk or cream in which they have grown.

Sweet cream delivered to dairy plants sometimes shows a ropy condition and, as with sweet milk, this is most likely to occur when the holding temperature is quite low. Ropiness is also met with rather frequently in sour cream and is occasionally observed in butter cultures; in these products it is usually due to the action of the organisms involved in the acid development.

Organisms important in causing ropiness in market milk. One of the more common of the ropy milk organisms is *Bacterium viscosum* which was probably first isolated by Adametz from water. After noting the action of the organism in milk and predicting that it might be the cause of ropiness in this product, Adametz[63] secured it from ropy milk. It has been repeatedly isolated in Europe and has also been found in many sections of the United States; in the work done at the Iowa Agricultural Experiment Station it was found to be the most common cause of the outbreaks of ropy milk studied. In old cultures there is less acidity than there was in the original milk but the reaction change with this organism occurs very slowly. *Bact. viscosum* is one of the ropy milk organisms that definitely requires air and, accordingly, when it is the causative organism in epidemics, the ropiness is often noted in the cream only; in pure cultures in skim milk there is frequently a membrane at the surface. The organism produces a large capsule that can be demonstrated best in very young cultures since, in older cultures, the capsules from different cells seem to unite and draw out into long threads, leaving the cells free or embedded in the capsular material, instead of each having a definite capsule. *Bact. viscosum* grows well at fairly low temperatures, such as 10° C. (50° F.), although growth is much faster at somewhat higher temperatures. At 37° C. (98.6° F.) growth takes place but ropiness in milk is not as pronounced as at lower temperatures.

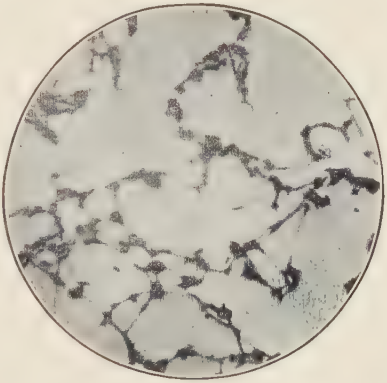


FIG. 9.—*Bact. viscosum* in very ropy milk, showing cells lying free or embedded in capsular material, instead of each being surrounded with a definite capsule, as is the case during the beginning of the growth of the organism in milk.

Buchanan and Hammer[64] studied an organism producing ropiness in milk which was essentially like *Bact. viscosum*, except that it eventually developed an acid reaction in milk; this organism was designated *Bacterium paraviscosum*.

Organisms of the Escherichia-Aerobacter group have been the cause of many epidemics of ropy milk in the United States; they have been found in Illinois, Iowa, Missouri, Wisconsin and undoubtedly other states. Most organisms classed with this group do not

[63] Milch-Ztg. 18. P. 941. 1889.

[64] Ia. Agr. Expt. Sta. Res. Bul. 22. 1915.

produce a ropy condition when grown in milk, but certain of them do; it is not unusual to isolate organisms of the latter class from dairy products which show no evidence of ropiness so it seems that they must be rather common. The Escherichia-Aerobacter organisms produce a ropy condition in milk by the development of capsular material that forms threads through the milk, as in the case of *Bact. viscosum.* Although the organisms produce gas in milk, the gas production is usually not observed under practical conditions, even when the milk is very ropy. The acid production of the organisms also seems to be of minor importance and milk is objected to on acount of the ropiness, without any mention of acid. At the Iowa Agricultural Experiment Station one of the Escherichia-Aerobacter forms was found to be the cause of ropiness in a lot of cream with an acidity of only 0.16 per cent.

Sadler and Middlemass[65] studied in detail a number of cultures secured from an outbreak of ropy milk and classed them as atypical *Escherichia neapolitana.*

In Europe much of the ropy milk is apparently due to coccus forms. While these seem to be of little importance in the United States, Hammer and Cordes[66] have reported an outbreak of ropy milk due to a coccus which they named *Staphylococcus cremoris-viscosi.* This organism rapidly digests milk but it develops a pronounced ropiness before the digestion is evident.

Organisms causing ropiness with considerable acid. Ropiness is frequently associated with the production of acid. The butter cultures carried in dairy plants may develop this condition after varying periods of successful propagation and ropy sour cream is occasionally encountered. Certain cultures of *S. lactis, L. casei* and *L. bulgaricus* show ropiness and this property seems to be lost or acquired with these organisms for no apparent reason, although in general a culture maintains either a ropy or non-ropy character. The ropy *S. lactis* cultures have been given the varietal designation, *S. lactis var. hollandicus.*[67]

With the cultures of the true lactic acid organisms that show ropiness this condition may be evident before any considerable amount of acid develops and then decrease as the acidity becomes pronounced. The ropiness is due to conditions other than capsule formation. *S. lactis* cultures frequently show some cells with capsules but the percentage of encapsulated cells is no larger with ropy cultures than with non-ropy ones. Ropy cultures of this organism sometimes contain long chains of cells and it seems that, in certain instances, these may be related to the ropiness, although cultures often show long chains without ropiness being evident and many ropy cultures show no chain at all. With certain *S. lactis* cultures ropiness appears to be due to large numbers of organisms; Hammer[68] has shown that some of these yield occasional non-ropy cultures when they are plated out and colonies picked. *L. bulgaricus* apparently does not produce capsules; the long chains of cells may tend to make the fermented milk draw out into threads, although rather long chains are present in some cultures that are not ropy. *L. casei* sometimes shows chains of cells but more often does not. This organism seems to produce capsules in both ropy and non-ropy cultures; there is some evidence that the number of organisms per cubic centimeter determines, to a large extent, the presence or absence of ropiness.

---

[65] Scientific Agr. 6. P. 297. 1926.

[66] Jr. Dairy Sc. 3. P. 291. 1920.

[67] See ref. 20.

Ropiness in whey. Ropy whey, in which the ropiness was due to *S. lactis,* was at one time used as a culture in the making of Edam cheese. A ropy condition due to *Bact. viscosum* has been observed at the Iowa Agricultural Experiment Station in the whey collecting when curdled milk was broken up in the manufacture of cottage cheese; such a relationship is unusual since this organism is not commonly found in the presence of considerable amounts of acid.

Ropiness due to associative action. Buchanan and Hammer[69] have reported a case of ropiness in cream that was due to the associative action of two organisms, one of which was *S. lactis.* The other organism, which was designated *Bacterium visco-symbioticum,* produced no change in milk in pure culture in three or four days but, when grown with certain *S. lactis* cultures, ropiness quickly developed. The ropiness produced by the combination seemed to be due to a large number of organisms since the plate method and microscopic observation showed that the mixed cultures contained much larger numbers of organisms than pure cultures of either type. The mixed cultures in milk also gave a slightly lower acidity than pure cultures of *S. lactis* and this may explain the greater multiplication of organisms in the mixtures. It is worthy of note that only certain cultures of *S. lactis* gave a ropy condition when grown in combination with *Bact. visco-symbioticum.*

[68] Ia. Agr. Expt. Sta. Res. Bul. 74. 1923.

[69] See ref. 64.

Ropy organisms of no practical importance. Many organisms from a wide variety of sources have been described as capable of producing a ropy condition in milk but most of these have never been found as the cause of epidemics and will likely never be of significance since they are so readily overgrown by other species. From the available data it seems probable that in the United States *Bact. viscosum* and the Escherichia-Aerobacter organisms are the important types from the standpoint of the economic losses due to ropy milk.

Nature of materials causing ropiness. The materials responsible for the ropiness developed in milk by bacteria are probably of two types, the true gums or gum-like substances and the mucins. The gums are polysaccharids while the mucins are nitrogenous mucus-like substances. It is probable that gums are more commonly the cause of ropiness in milk than the mucins; with certain organisms the gums produced seem to be galactans.

Sources of ropy milk organisms. In any epidemic of ropy milk the source of the responsible organism is one of the important considerations. After the organism is once established, infection undoubtedly occurs from the utensils, stables, etc., but the sterilization of containers, the cleaning of stables and other measures are likely to give only temporary relief, unless the original source is controlled.

The extensive studies that have been made of the flora of the interiors of normal udders have shown that ropy milk organisms are not ordinarily found there, and it is evident that, in general, these organisms come from external sources. However, organisms have been secured from infected udders that were capable of producing ropy milk and in an outbreak described by Hammer and Cordes[70] *Staph. cremoris-viscosi* was found in milk drawn so as to exclude contamination from outside the udder, although it was impossible to say whether the udder invasion preceded or followed the epidemic.

In epidemics of ropy milk, the organisms often come from surface waters. One of the common routes of infection is the contamination of the coats of the cows from which the organisms then fall into the milk pail at milking time. Harding and Prucha[71] reported that, in two instances in connection with an outbreak studied by them, material collected from the flanks and udders of cows produced characteristic ropiness when put into sterile milk. Epidemics of ropy milk have been known to promptly follow the flooding of the lands on which the producing animals were pastured, and there have been a number of outbreaks stopped by simply keeping the animals away from surface waters. In this connection it is of interest to note again that *Bact. viscosum* was isolated from water before it was secured from ropy milk. In addition to water standing in pastures, that from troughs, milk cooling tanks, etc., must be considered as possible sources of the organisms.

---

[70] See ref. 66.

[71] Ill. Agr. Expt. Sta. Bul. 228. 1920.

When ropy milk organisms get into the utensils they may live over from one milking to the next with the methods of caring for utensils that are practiced on many farms. Harding and Prucha[72] noted that samples collected on farms delivering milk containing ropy organisms showed the presence of this type in the utensils; at one farm the organisms were repeatedly found in the utensils and in the water of the cooling tank. Ward[73] found *Bact. viscosum* the cause of ropiness in two creameries in both of which the organism appeared to come from the use of insufficiently scalded utensils. Greater care in the scalding brought the trouble to an end. In one outbreak three different quantities of sterile milk picked up the organism from the surface of the strainer.

Controlling ropiness. In stopping an outbreak of ropy milk the sterilization of all utensils and equipment coming in contact with the milk, including bottles and cans, is one of the first steps. The cooling tank may be a source of the organisms through water splashing from it into the milk. The stables should be given a good cleaning and disinfection since, in some instances, the milk is apparently contaminated from the air of the stables. The flanks and udders of the cows may be wiped with a cloth dampened with a disinfecting solution and the continued contamination of the coats of the animals prevented by keeping them away from surface waters. The control of an epidemic is not an easy matter and there is likely to be a recurrence when the precautions taken are neglected, even for a short time. In certain cases ropy milk has been eliminated only after a long period of careful attention to a great many details. The pasteurization of the milk is a practice that usually gives immediate relief but, in some instances, there have been outbreaks in pasteurized supplies; with smaller dealers not regularly pasteurizing, however, the use of this process commonly makes it possible to immediately prevent ropiness and thus retain customers that would be lost if the ropiness continued.

Heat resistance of the common ropy milk organisms. Harding and Prucha[74] investigated the heat resistance of a number of cultures isolated from outbreaks of ropy milk, including both the Escherichia-Aerobacter and *Bact. viscosum* types. When heated in bouillon, some of them survived 60° C. (140° F.) for 10 minutes but not 65° C. (149° F.) for this period. In ropy milk a number survived 60° C. for 20 minutes but not for 30 minutes. These results indicate that proper pasteurization will destroy the organisms commonly responsible for ropy milk.

---

[72] See ref. 71.

[73] N. Y. (Cornell) Agr. Expt. Sta. Bul. 165. 1899.

Some very extensive outbreaks of ropy milk have occurred, however, with pasteurized supplies. In general, these have been due to contamination from the plant equipment after the heating and it is probable that bottle fillers have been most often involved because of the difficulty of thoroughly sterilizing them; the ropy milk organisms may undoubtedly survive inefficient pasteurization in some instances. The outbreaks in pasteurized milk can be stopped by a thorough cleaning and disinfection of the plant and equipment, but a recurrence of the difficulty is frequently noted.

Isolation of the causative organism. The isolation of the causative organism from a sample of ropy milk or cream can usually be accomplished by plating when the condition is due to such organisms as *Bact. viscosum* or one of the Escherichia-Aerobacter forms. With these types, the viscous character of the colonies makes them easily detected in a mixed flora. If the ropiness is thought to be due to *S. lactis* it is advisable to use whey agar for plating in addition to beef extract or beef infusion agar. Some of the plates poured for the isolation of ropy milk organisms should be incubated at room temperature since, with *Bact. viscosum* and the Escherichia-Aerobacter types, the colonies are likely to be less ropy at 37° C. (98.6° F.) than at lower temperatures. Such organisms as *L. bulgaricus* and *L. casei* are often isolated only with difficulty because of their poor growth on agar.

Distribution of the ropy milk organisms. The common presence of ropy milk organisms, even when ropiness does not develop in the milk, has been shown by Ward[75] who found that the milk furnished by 42 patrons, out of a total of 307 delivering milk to three plants, became ropy when held for four days at 4.4° to 7.2° C. (40° to 45° F.); no complaints of ropy milk were received at the plants. This investigator noted that, in several plants, ropy milk organisms were frequently present in any utensil that had been washed, but not

[74] Jr. Dairy Sc. 3. P. 502. 1920.

[75] Jr. Dairy Sc. 6. P. 616. 1923.

scalded, and in various pieces of equipment. Harding and Prucha[76] reported that the holding of individual samples, from the 140 farms supplying a bottling plant having trouble with ropy milk, yielded ropiness one or more times with the milk coming from 116 of the farms.

Organisms which will produce ropiness in milk are often isolated from milk and cream which are either normal or which show abnormal conditions other than ropiness. It is evident that ropy milk organisms may be present in these products without causing a change in consistency; this is due to their overgrowth by other types.

Use of ropy milk. Ropy milk resulting from the growth of *L. bulgaricus* has been added to buttermilk which "wheys off" rapidly to delay the settling of the casein. Some of the fermented milks consumed in certain countries, particularly the Scandinavian countries, are ropy and while these milks are not prized because of the ropiness, this condition is not objected to. Macy[77] has isolated a Streptococcus producing ropiness in milk from a ropy milk beverage used by certain of the Finns in the United States.

THE GASSY FERMENTATION

The development of gas in milk, cream, etc., through the action of micro-organisms is referred to as the gassy fermentation. This change may be brought about by a variety of organisms but the number of species that are of practical significance is small. The composition of the gas evolved is variable, depending on the organism responsible, but carbon dioxide is ordinarily one of the prominent constituents.

Importance of the gassy fermentation. The formation of gas in milk and cream is unusual when these products are in a sweet condition and, accordingly, the fermentation is of little importance in the market milk industry. In sour cream it is frequently observed, especially during the warm months when the cans on the receiving floors of butter plants may show a vigorous gas evolution. It is not an unusual occurrence to have the covers blown to the ceiling and the cream "foam" or "boil" out when the wires holding on the lids are clipped; this is particularly true when the cream has been in transit or on the producing farms for considerable periods. Cans in express cars or on railroad platforms may show cream foaming out around the lids, due to the active gas formation within. This foaming cream is often referred to as "yeasty" because of the pronounced yeasty odor, and investigations of the condition by Hunter[78] and by Hammer and Cordes[79] have shown that the causative organisms are torulae. With this fermentation there is a loss due to the cream that foams out of the cans and another as a result of the decreased quality for buttermaking purposes.

---

[76] See ref. 74.

[77] Jr. Dairy Sc. 6. P. 127. 1923.

The development of gas sometimes occurs in milk being fermented in the making of such products as cottage cheese and butter culture. Escherichia-Aerobacter species are responsible in certain of these cases, while in others the torulae are involved. Whey standing in vats or cans occasionally undergoes a gassy fermentation which is most often due to the torulae. This change is favored by the relatively high temperatures at which whey is often allowed to stand.

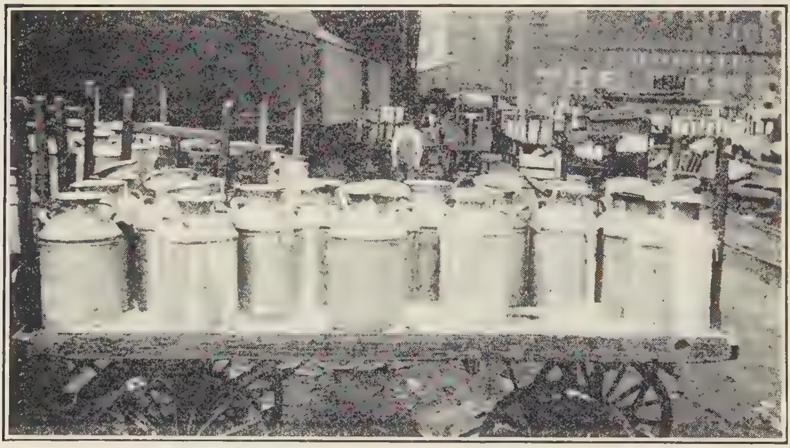


FIG. 10.—Yeasty cream on a railroad platform. Courtesy Dairy Record.

The torulae. The torulae of importance in causing the development of gas in dairy products are of two types, *Torula cremoris* and *Torula sphaerica*.[80] The important characteristic of these organisms is their ability to produce carbon dioxide and small amounts of ethyl alcohol from lactose; because of their acid tolerance they often grow in materials in which the acidity prevents the growth of many other organisms. The striking differences between these two species are in the shape and size of the cells and in the growth temperatures;

[78] Jr. Bact. 3. P. 293. 1918.

[79] Ia. Agr. Expt. Sta. Res. Bul. 61. 1920.

[80] See ref. 79.

T. cremoris has the ellipsoidal shape so common with yeasts and grows better at 37° C. (98.6° F.) than at lower temperatures while, with *T. sphaerica*, most of the cells are essentially spherical and there is often little or no growth at 37° C., the optimum temperature being somewhat lower. Both organisms produce a vigorous gas evolution and a yeasty odor in litmus milk at favorable temperatures. A reddening of the litmus also occurs and, in old cultures, there is some-

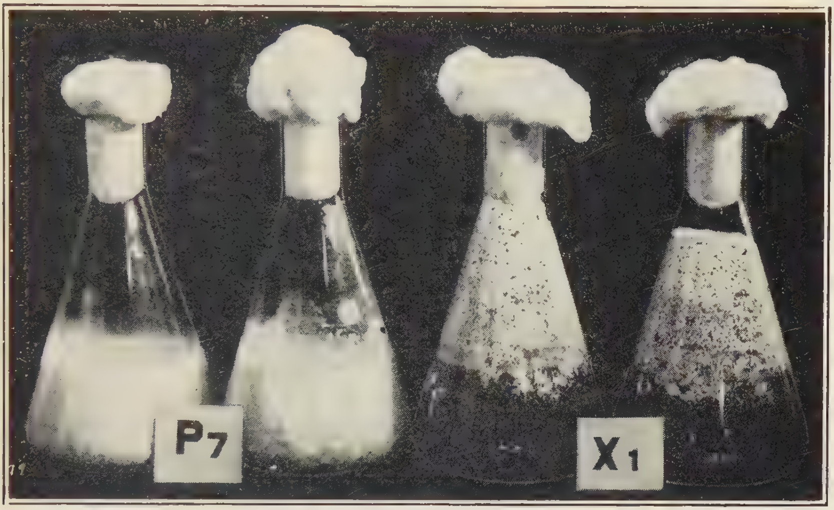


Fig. 11.—Types of gas formation in milk.

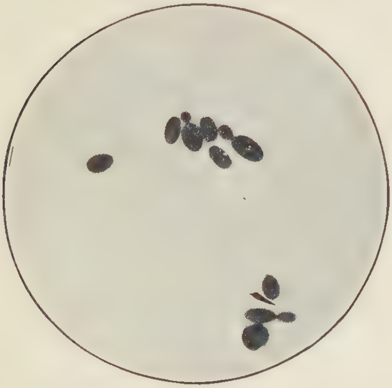


Fig. 12.—*Torula cremoris.*

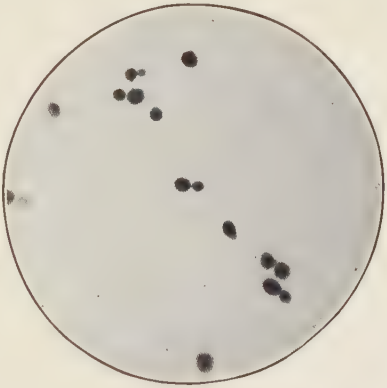


Fig. 13.—*Torula sphaerica.*

times coagulation, especially in the lower portion. The organisms grow well on agar and give a raised, smooth edged, shiny, chalk white growth, having a yeasty odor.

Development of T. cremoris and T. sphaerica in cream. *T. cremoris* and *T. sphaerica* are commonly present in cream, either sweet or sour. The growth of these organisms is greatly favored by comparatively high temperatures and, accordingly, their rapid development is largely limited to the warm months. Occasionally a lot of cream that is held in a heated room may show yeastiness during the winter but the fermentation is decidedly a seasonal one and quite strictly confined to the summer. The ability of the organisms to grow in the presence of considerable amounts of acid makes possible their development in cream which has a high acidity as a result of the extended activity of the acid producing organisms.

In pure culture the action of *T. cremoris* or *T. sphaerica* is not such as to produce a typical foamy condition in milk or cream. Without coagulation there is little tendency for the gas formed to be held and it readily escapes. However, when coagulation has occurred, the gas tends to be retained and the volume of the fermenting material is increased by the gas bubbles held. In both sweet and sour cream there is commonly a supersaturation with gas and, when this comes out of solution, due to jarring or agitation, it readily escapes from uncoagulated milk or cream while, with the coagulated products, it is partly held and yields a foamy mass. The typical foaminess in cream accordingly seems to be due to associative action, the torula forms producing the gas and some other type causing the necessary coagulation by the development of acid.

It is impossible to say whether *T. cremoris* or *T. sphaerica* is the more important as a cause of foaminess in cream. The preliminary report of Hammer and Cordes [81] seemed to indicate that *T. sphaerica* is comparatively rare but this was probably due to using 37° C. (98.6° F.) for incubating the isolation plates, at which temperature *T. sphaerica* does not grow well. More recent work at the Iowa Agricultural Experiment Station has shown that both organisms are very common and can often be isolated from the same sample of cream.

Control of yeastiness in cream. The control of yeastiness in cream logically lies in the direction of using low temperatures to prevent the growth of the responsible organisms. Since these species are found in cream regularly, even in that coming from farms that have never had trouble with foaminess, it appears that they can not be kept out of cream with the ordinary methods of production. Proper holding temperatures have repeatedly proved efficient in preventing the development of foaminess in areas where the condition is very common.

---

[81] See ref. 79.

When cream must be subjected to rather high temperatures for considerable periods, the use of pasteurization makes it possible to prevent foaminess and this practice has been employed effectively in shipping cream from cream stations. It can not be economically adopted in shipping cream from individual farms because of the small volumes involved.

Isolation of T. cremoris and T. sphaerica. One or both of the common lactose fermenting torulae can ordinarily be secured from a sample of milk or cream by allowing it to stand at approximately 30° C. (86° F.) until the odor and gas production suggest that there has been a good growth of the desired organisms and then plating on some such medium as whey agar. The addition of a small amount of acid, for example, one cc. of a one per cent solution of tartaric acid crystals for ten cc. of medium, to the plates at the time of pouring prevents the growth of many types of bacteria and thus simplifies the isolation.

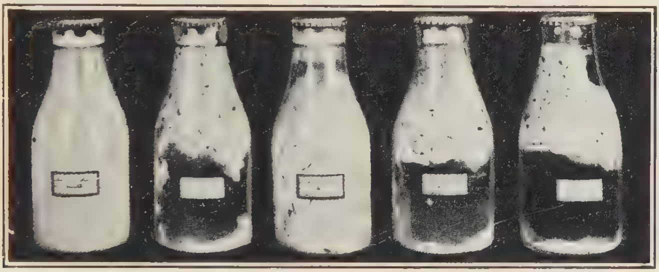


Fig. 14.—Associative action in the formation of gas in milk; bottle at left shows the action of the gas former alone, while remaining bottles show the action of the gas former in combination with *Streptococcus lactis*. Iowa Agr. Expt. Sta. Res. Bul. 27.

Gas production by the Escherichia-Aerobacter organisms. The source of the gas formed in milk and cream by the Escherichia-Aerobacter organisms is also the lactose. Ordinarily, in pure cultures, the most active gas evolution precedes any considerable acid production and the gas readily escapes from the uncoagulated milk while, in coagulated milk, the gas tends to be held. In milk inoculated with butter culture for the manufacture of cottage cheese, Escherichia-

Aerobacter forms have been found the cause of extremely gassy conditions, the curd being full of gas holes and sometimes projecting several inches above the top of the vat. Hammer [82] isolated an Escherichia-Aerobacter species from such material and noted that, when grown alone, it produced abundant gas but no typical gassy curd while, when inoculated along with *S. lactis,* a very gassy curd developed.

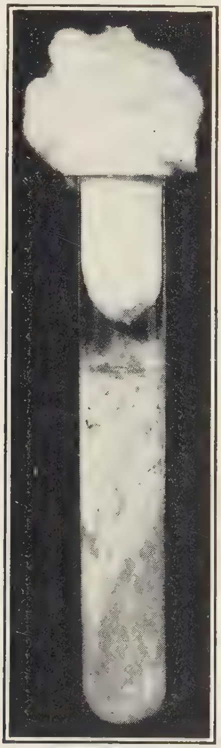


FIG. 15.—Action of an anaerobic gas former in milk.

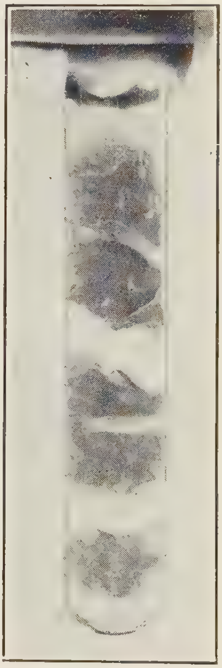


FIG. 16.—Anaerobic gas former in agar shake culture.

Gas formation in butter cultures. Gas production in butter cultures may be caused by a number of different organisms, including both bacteria and torulae. Although in certain instances the gas forming organisms may be eliminated by repeated transfer, attempts along this line often fail and gassy butter cultures should ordinarily be discarded.

---

[82] Ia. Agr. Expt. Sta. Res. Bul. 27. 1916.

A very few small gas bubbles in butter cultures are sometimes the result of the activity of the flavor and aroma producing organisms associated with *S. lactis*. This type of gas formation should not be considered objectionable; it is only occasionally evident and is always slight so that it is not likely to be confused with the gas formation due to contaminating organisms.

Gas formation with the spore forming anaerobes. Some of the spore forming anaerobes produce gas in milk and cream, especially when the non-spore forming organisms are destroyed by heat. There are two general types of these anaerobes, (1) the putrefactive forms and (2) the non-putrefactive forms; the latter type can be divided into (a) the organisms producing primarily acids, such as propionic and butyric, in milk and (b) the organisms producing primarily butyl alcohol and related compounds. The anaerobes are occasionally responsible for gas formation in dairy products but are greatly exceeded in importance by other types of organisms.

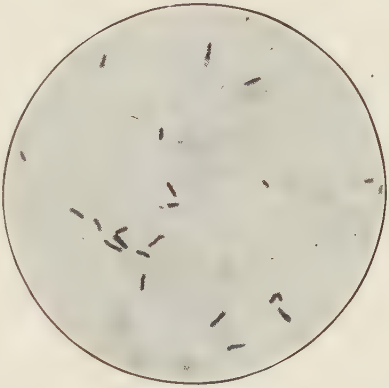


FIG. 17.—Anaerobic gas former in milk.

THE SWEET CURDLING FERMENTATION

The sweet curdling fermentation is the process by which microorganisms curdle milk or cream without the development of sufficient acid to bring about the coagulation. The immediate cause of the curdling is the production, by the micro-organisms, of enzymes whose properties are comparable to those of rennin. Some of the sweet curdling organisms produce little or no acid while others form considerable amounts after the curdling has taken place at a low acidity.

Importance of the sweet curdling fermentation. Sweet curdling is one of the more uncommon fermentations although it is by no means rare. The milk and cream delivered to dairy plants occasionally show this condition and it is sometimes observed by the retail purchaser of these products, especially when they are held for considerable periods after pasteurization. Sweet curdling is most frequently encountered during the warm months or when the product involved is kept at a fairly high temperature.

Cream showing a normal acidity has been observed to coagulate during pasteurization in butter plants and this has been assumed to be due to the presence of small amounts of a coagulating enzyme whose activity is greatly increased at a comparatively high temperature. The work of Frazier[83] confirms this idea. He found that, although small amounts of a milk culture of a sweet curdling organism inhibit heat coagulation over a small range, large amounts hasten the heat coagulation. When a purified bacterial rennin was used there was no influence on the heat coagulation when the milk was heated immediately upon the addition of the enzyme but, when the rennin was allowed to act for a time, the heat coagulability of the milk was increased. The optimum temperature for most rennins was found to be 40° to 43° C. (104° to 109.4° F.) while 60° C. (140° F.) for 15 minutes destroyed them.

Although the sweet curdling fermentation is not commonly met with under practical conditions, almost any lot of milk contains organisms capable of bringing about this change. The failure of these to develop is due, ordinarily, to their overgrowth by the lactic acid organisms and it takes unusually favorable conditions, with perhaps small numbers of competing species, for sweet curdling to occur.

Causative organisms. The organisms that are capable of sweet curdling milk are extremely varied in their characteristics and belong to widely separated genera; the general types are as follows:

1. *The aerobic spore producing bacteria.* The aerobic spore producing bacteria include a number of species that are pronounced sweet curdlers. Organisms of this type are regularly present in milk and cream and can be easily isolated by heating to destroy the non-spore formers, holding under aerobic conditions at a temperature favorable for growth, such as 25° to 30° C. (77° to 86° F.), until curdling occurs and then plating on standard or beef infusion agar. In general these organisms get into milk from dust, dry manure, etc., the spores enabling them to resist the desiccation to which such materials are subjected. The aerobic spore producers are not commonly the cause of sweet curdling under practical conditions but may be in some instances, especially in pasteurized milk or cream held at a comparatively high temperature.

[83] Jr. Dairy Sc. 8. P. 370. 1925.

Lawrence and Ford[84] studied the aerobic spore formers in the Baltimore milk supply and reported that they believed *Bacillus albolactus* to be the common cause of the clotting and peptonization occasionally seen with boiled milk and also that it is undoubtedly a contributing factor in the changes seen in milk heated to temperatures such as 60° to 65° C. (140° to 149° F.) This organism was encountered less frequently in samples of milk heated with various exposures than either *Bacillus cereus* or *Bacillus subtilis.*

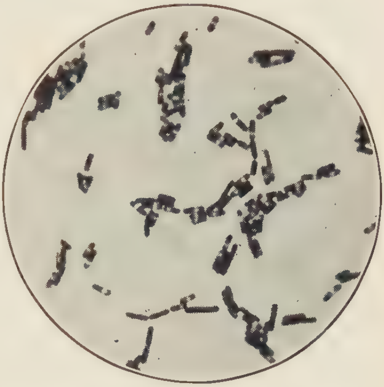


FIG. 18.—Spore forming sweet curdler.

2. *The non-spore producing rods.* A number of non-spore producing rods are capable of sweet curdling milk. With most of them the curd formed is very soft and inconspicuous and the striking thing is a proteolysis of the casein. The organisms belonging to the genus Proteus are representative of this general group and illustrate the usual type of change in milk. The non-spore producing rods that cause sweet curdling are frequently encountered in studying the bacterial flora of milk and cream and apparently come from a wide variety of sources; they are rarely, if ever, responsible for sweet curdling under practical conditions.
3. *The coccus forms.* Various species of cocci with widely differing characters can also sweet curdle milk. Perhaps the most important among these is the rennin acid form, *Streptococcus liquefaciens,* which coagulates milk and cream by the production of an enzyme and then produces considerable amounts of acid so that the final acidity may be as high as 0.8 or 0.9 per cent; later there is an extensive proteolysis of the casein. This organism was found to be responsible for sweet curdling in a number of instances by the Iowa Agricultural Experiment Station and the curdling was usually accompanied by the development of a bitter flavor; in one or two cases the bitterness

---

[84] Jr. Bact. 1. P. 273. 1916.

was the cause of the samples being submitted for examination and the curdling was not particularly noticeable. The acid produced by a number of *S. liquefaciens* cultures studied was found to include considerable quantities of lactic acid; the isomeric form of this was variable, both *d* and *i* acid being noted. *S. liquefaciens* has the same general morphology as *S. lactis* and is also quite like it in cultural characters other than the proteolysis of casein.

Some of the micrococci commonly found in milk can also sweet curdle it, but bring about the change rather slowly and, accordingly, are unimportant as a cause of this fermentation under practical conditions.

PROTEOLYSIS

Proteolysis is the process by which casein or some insoluble casein derivative is broken down to water soluble compounds through the action of micro-organisms. The extent of the decomposition varies greatly with different species; sometimes it is almost complete and is readily evident, while in other instances it is slight and can be definitely established only by a chemical determination of the amount of

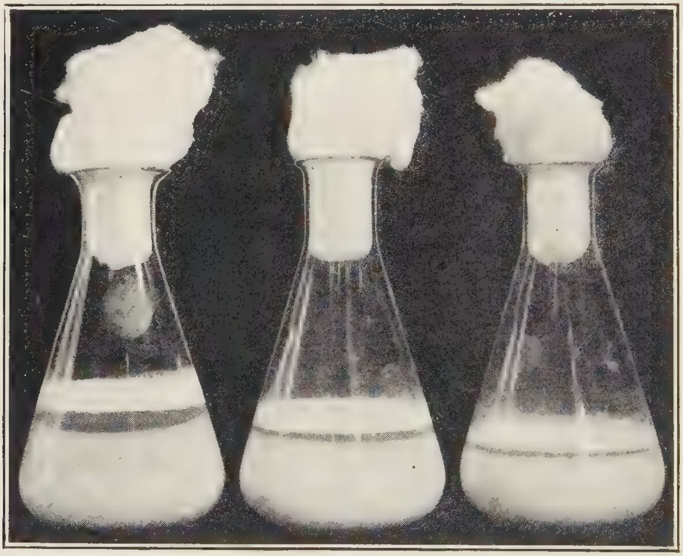


Fig. 19.—Proteolysis following sweet curdling. The undigested surface portions are cream.

soluble nitrogen. The decomposition products formed are usually a mixture of various groups of bodies and include peptones and related compounds, amino acids, ammonia and, occasionally, free nitrogen.

Relationship of proteolysis and sweet curdling. The proteolysis of milk and cream quite regularly follows the sweet curdling and it seems that these two changes are very closely related. Experimental data show that, with certain organisms at least, the incubation temperature used determines whether the proteolysis or the sweet curdling is the more evident. Sarkaria and Hammer[85] noted that, with the organisms studied by them, coagulation was more conspicuous at 37° C. (98.6° F.) than at room temperature. The observations of various investigators, including those of Frazier,[86] suggest that some of the organisms which sweet curdle milk when first isolated lose this property on cultivation and then have increased proteolytic powers. However, the effect of various salts on the activity of bacterial rennin, as pointed out by Frazier, may be a factor tending to cause variations in the results obtained with an organism.

The frequency with which the proteolysis and sweet curdling of milk are associated in the characters of micro-organisms has led to the suggestion that they always occur together and that, occasionally, one of these changes is overlooked in studying an organism because it is not pronounced at the temperature used. Cultures that have been described as proteolyzing milk without any mention of sweet curdling sometimes show a very definite and complete sweet curdling when grown at suitable temperatures. It is of interest to note that the association of milk proteolysis and coagulation in the characters of micro-organisms is in agreement with their association in the intestinal tracts of various animals.

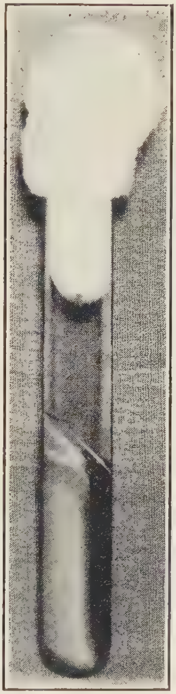


FIG. 20.—*Streptococcus liquefaciens* in milk, showing proteolysis following coagulation.

Bitterness following proteolysis. The proteolysis of milk and cream is often accompanied by the development of a bitter taste. This is due to the presence, among the protein decomposition products, of such bodies as peptones, etc. that have a bitter taste. Sometimes bitterness is evident in milk or cream before either proteolysis or sweet curdling are conspicuous. Under practical conditions it is most frequently observed when these products are held at fairly low temperatures, such as 10° C. (50° F.), and presumably occurs as a result of the slow growth of *S. lactis* at these temperatures. *S. liquefaciens* is one of the more common organisms causing bitterness.

---

[85] Jr. Dairy Sc. 11. P. 89. 1928.

[86] See ref. 83.

THE FLAVOR AND AROMA FERMENTATIONS

Various changes in the flavor and aroma of milk and cream are brought about through the action of micro-organisms. Flavor and aroma are so interrelated that they are necessarily considered together; what is often taken to be the flavor of milk or cream is, in reality, a combination of the flavor and aroma.

Importance of the flavor and aroma fermentations. Changes in the flavor and aroma of milk and cream, as a result of bacterial action, are very common. The milk coming to market milk plants, cheese factories and condenseries is usually examined for the presence of off flavors and aromas, many of which are bacterial, and certain of the butter factories also have high standards for the raw material received by them. In sour cream which has been kept for considerable periods at fairly high temperatures, there are frequently very pronounced flavors and aromas due to micro-organisms. These are the cause of serious economic losses since the quality of the butter made from cream containing them is certain to be more or less unsatisfactory, even when attempts are made during the manufacturing process to eliminate the undesirable conditions, and the price received is correspondingly low. In some instances the bacterial flavors and aromas present in milk and cream are so objectionable that the products are refused at dairy plants.

Abnormal flavors and aromas not due to micro-organisms. There are various factors, other than the activity of micro-organisms, that may be responsible for abnormal flavors and aromas in milk and cream. These are of importance in a consideration of the flavor and aroma fermentations since it is necessary to know the cause of an abnormal condition before an intelligent attempt at its correction can be made. The following grouping includes most of the causes of abnormal flavors and aromas of milk and cream, other than micro-organisms.

1. Absorption of odors.—Odors are quickly absorbed from the surroundings by exposed milk and cream and this absorption

may markedly influence the flavor and aroma. Absorption is a common cause of such flavors and aromas as gasoline, fly-spray, disinfectants, cellar, etc., and may play a part with others, such as feedy and barny flavors and aromas. It was at one time commonly believed that milk would not absorb odors as long as it was warmer than the surrounding atmosphere but it is now generally recognized that absorption occurs whether the milk is warm or cold. The rapidity with which milk can absorb odors is shown by the investigations of Russell;[87] horse manure was absorbed in one-fourth hour, cow urine in one-half hour and silage in one hour. It seems certain that, in many instances, a disagreeable flavor and aroma are, in part, due to the absorption resulting when milk is allowed to stand in stables, the air of which is saturated with the odors of manure, silage, etc. The fat is the constituent of milk and cream mainly involved in this absorption.

2. Materials consumed by the cows.—The materials consumed by the producing animals may play an important part in determining the flavor and aroma of milk and cream. Feed flavors and aromas are imparted to the milk through the bodies of the cows and certain feeds, such as silage, alfalfa, turnips, etc., are responsible for definite abnormal conditions. Drugs and drinking water having pronounced flavors and aromas may act in the same way. When the materials are fed shortly before milking the flavors and aromas due to them are more pronounced than when a longer period elapses and, if the time is extended enough, they are often not noticeable, unless large amounts have been given. Variations occur in the intensity of flavors and aromas in the milk of different animals receiving the same materials under the same conditions.

Gamble and Kelley[88] found that silage fed one hour before milking affected the milk and suggest that silage should be fed immediately after milking. In their experiments not over 15 to 25 pounds of corn silage could be fed twice daily after milking without imparting a discernible flavor and aroma to the milk. In trials reported by Babcock,[89] feeding green alfalfa to dairy cows at the rate of 30 pounds per cow one hour before milking, produced very pronounced off flavors and aromas in

[87] Wis. Agr. Expt. Sta. 15th An. Rpt. P. 104. 1898.

[88] U. S. D. A. Bul. 1097. 1922.

[89] U. S. D. A. Bul. 1190. 1923.

the milk while with 25 pounds of green corn fed one hour before milking the off flavors and aromas were so slight they would seldom be noticed by the average consumer. Babcock[90] also found that turnips fed to dairy cows at the rate of 15 pounds per cow one hour before milking produced objectionable flavors and aromas in the milk. This investigator[91] has reported the detection of a garlic flavor and aroma in milk when the samples were taken one minute after feeding garlic; the intensity of the garlic flavor and aroma increased as the interval between feeding the garlic and milking increased, until after ten minutes it was very pronounced. A strong garlic flavor and aroma were found in milk drawn two minutes after the animals had inhaled the odor of garlic for ten minutes. A garlic odor was evident in the samples of blood drawn after the feeding of garlic. Studies on the influence of various other feeds on the flavor of milk have also been carried out by Babcock.[92, 93 and 94]

Certain of the objectionable feed flavors are largely seasonal. A garlic flavor is most common in the spring because garlic is one of the first plants to start growth and the animals then eat it readily, but it may occur at other times. Certain weed flavors are more frequently encountered in the late summer when the pastures are dry and the cows eat plants that were rejected earlier, when feed was abundant. Feed flavors are often more pronounced in cream than in the milk from which it came, due to the pronounced absorbing power of the fat.

The effect of feed on the flavor of milk is sometimes very evident when the cows are kept on one type of pasture during the day and another during the night. In one instance noted at the Iowa Agricultural Experiment Station the evening milk from a certain farm had a pronounced legume flavor while the morning milk did not. An inquiry led to the information that the cows were on sweet clover during the day but were kept nearer the barn on June grass during the night.

Aeration seems to be helpful in removing many feed flavors and aromas from milk and cream, although when they are pronounced the process may not completely eliminate them.

It should be recognized that flavors and aromas in milk due

[90] U. S. D. A. Bul. 1208. 1923.

[91] U. S. D. A. Bul. 1326. 1925.

[92] U. S. D. A. Bul. 1297. 1924.

[93] U. S. D. A. Bul. 1342. 1925.

[94] U. S. D. A. Tech. Bul. 9. 1927.

to the materials consumed by the producing animals represent instances of absorption before the milk leaves the udder. The constituents responsible for the flavors and aromas are probably carried to the udder through the blood and lymph.

3. Condition of the cows.—There are a number of conditions in the producing animals that may affect the flavor and aroma of the milk. When a cow is advanced in lactation the milk frequently shows an undesirable flavor that is commonly described as salty, bitter or rancid and a salty or bitter flavor may be present in milk coming from infected udders. With both of these conditions there is a great deal of variation among different animals in the extent of the undesirable flavor and it may even be entirely lacking.

   The flavor present in milk, as a result of advanced lactation in the producing animals, is often not noticed until the milk has stood for a time. After it is evident it usually increases up to a certain point with the age of the milk. Eckles and Shaw [95] reported the flavor of the milk of four animals advanced in lactation as rancid and bitter combined and stated that it developed within 24 hours. Palmer [96] found this abnormal flavor due to the activity of a lipase which hydrolyzes the fat with the liberation of fatty acids; the lower volatile fatty acids, especially butyric, impart, in large measure, the objectionable flavor and aroma. The enzyme is active, even at fairly low temperatures, but can effectively be retarded by heating to 75° C. (167° F.) for a few minutes. In advanced lactation there is commonly an abnormally high chlorine content which may be, in part, responsible for the abnormal flavor since this is frequently described as salty. The chlorine content of milk does not always rise with advancing lactation, however; Trunz [97] studied the composition of the ash of the milk of two cows throughout the lactation period and found that, with one, the chlorine varied only from 11.5 to 12.0 per cent while, with the other, it increased from 11.86 to 24.77 per cent.

   The off flavor in the milk of animals with infected udders is presumably due to variations in the composition, which occur as a result of the condition of the udder tissues.

   It seems that the individuality of the animal may also be of importance from the standpoint of the flavor and aroma of the milk, entirely aside from such factors as the feed consumed, the stage of lactation, etc. Certain cows, even under very favorable conditions, give milk with more or less of an unpleasant flavor and this may continue through succeeding lactation periods. It also appears probable that, with some animals, the flavor due to advanced lactation occurs very much earlier in the lactation period than with others. Conversely, the milk of certain cows seems to have a particularly pleasing flavor and while this may be in part due to its richness, other factors are also involved.

---

[95] U. S. D. A. B. A. I. Bul. 155. 1913.

[96] Jr. Dairy Sc. 5. P. 201. 1922.

[97] Referred to in ref. 95.

In connection with the influence of the condition of the animal on the flavor of the milk it should be pointed out that there may be differences in the flavor of the milk from the various quarters and also from various portions of the milking. The fore milk sometimes has a rather pronounced unpleasant flavor and the strippings may have either a less pleasant or a more pleasant flavor than the main portion of the milking, depending undoubtedly on whether or not the off flavor is one associated with the fat.

The flavors and aromas due to the condition of a producing animal are less often noted in the milk from herds than in the milk from individual cows since, with the herd milk, an off flavor in the milk of any one animal is more or less obscured by the mixing of the milk. However, these flavors and aromas sometimes involve considerable volumes of milk and cream.

4. Action of sunlight—When milk and cream are exposed to sunlight, they rather quickly acquire an off flavor and aroma which, when well developed, suggest tallow and are often referred to as tallowy. The condition is most often encountered in bottled milk left on the porch for some time after its delivery. Hammer[98] noted an off flavor in milk exposed to sunlight for only 10 minutes while definite tallowiness was observed with exposures as short as 45 minutes. Exposed milk had a chalky appearance and, when churned, yielded a lighter colored fat than the unexposed milk. Tallowiness develops on exposure to sunlight at very low temperatures and has been noted at the Iowa Agricultural Experiment Station in ice cream held at such a low temperature that it remained firm.
5. Addition of foreign materials.—Foreign materials are occasionally added to milk and cream and the flavor and aroma influ-

---

[98] Ia. Agr. Expt. Sta. Res. Bul. 64. 1920.

enced in this way. Soap may be left in a utensil at the time of washing, or a pail or can may be employed in transporting some such material as gasoline, kerosene or oil, and then used for milk or cream without proper cleaning.

General types of organisms causing flavor and aroma fermentations. The number of organisms that produce changes in the flavor and aroma of milk and cream in which they grow is very large; many of those involved in the fermentations already discussed do this and it is probable that most of the organisms that multiply in milk and cream affect the flavor and aroma to some extent. Certain organisms capable of growth in milk and cream are of no importance from the standpoint of multiplication under practical conditions, because of the competition which they meet, and those which grow actively are the ones primarily concerned with the flavor and aroma changes. The micro-organisms that are responsible for these fermentations include the bacteria, yeasts and molds; ordinarily the yeasts and molds become of significance only after milk and cream have been held for a considerable period.

Types of flavors and aromas produced. The flavors and aromas produced in milk through the action of micro-organisms are extremely varied. Undoubtedly an acid flavor and aroma is the most common but many others are frequent. Flavors and aromas, such as feedy or barny, that suggest other causes may be due to organisms. In an instance studied at the Iowa Agricultural Experiment Station the milk delivered by a certain producer frequently showed a flavor that was thought to be due to a feed, but which investigation proved was the result of the growth of an organism; the flavor was common in the night milk that was held for a time on the producing farm but was absent in the morning milk that was delivered soon after milking. Barny and other objectionable flavors may apparently be due to the growth in milk and cream of the organisms which are responsible for decompositions in manure. Such specific flavors as soapy and turnipy are, in occasional instances, due to the activity of organisms instead of to the expected causes.

A malt-like flavor and aroma is often present in milk and cream as a result of the growth of *S. lactis var. maltigenes*[99] and may be regularly noted in the product from a certain farm. Bitterness appears to be due to a variety of organisms, including *S. liquefaciens.*

---

[99] Ia. Agr. Expt. Sta. Res. Bul. 68. 1921.

Harrison[100] found that a bitter condition in milk delivered to a cheese factory was caused by a lactose fermenting torula, which he named *Torula amara.* Often the combined growth of various organisms results in an off flavor and aroma that is readily recognizable but which has no outstanding character and is designated as unclean. Organisms producing fishiness in milk and cream have also been isolated. Yeasty and moldy flavors and aromas are especially common in the old acid cream which is delivered to butter plants after careless methods of production and handling.

Many of the off flavors and aromas produced in milk and cream are rather temporary and are replaced by others that may be still more objectionable. When the holding period is extended, there are certain sequences that occur with considerable regularity; for example, a fishy flavor and aroma in cream is commonly followed by a metallic flavor.

The temperature of holding has a great influence on the flavors and aromas that develop. Temperatures such as 0° C. (32° F.) largely prevent the growth of *S. lactis* and thus permit the activity of certain organisms, sensitive to acid, that are capable of causing pronounced protein decomposition. Small differences in the temperatures of holding often result in wide differences in the flavors and aromas produced, because of the variations in the comparative growth of the organisms present.

The flavors and aromas produced in milk and cream by micro-organisms are occasionally very pleasant, although decidedly abnormal; fruity flavors and aromas are perhaps the best examples. In general, these pleasant flavors and aromas are quickly replaced by others that are objectionable.

Associative action. With the flavor and aroma fermentations, as with so many other changes in milk and cream, the associative action of organisms is important. Flavors and aromas that, from their manner of development, are evidently the result of bacterial action sometimes can not be reproduced with pure cultures while mixed cultures yield the condition. The difficulties encountered in attempting to develop certain flavors and aromas experimentally, suggest that the balance between the causative organisms is often a delicate one. Anaerobes that can not grow in milk and cream in pure culture may develop in impure culture through the action of other types in using up the oxygen present. The study of the flavors and aromas due to micro-organisms is greatly complicated by the necessity of sterilizing the milk and cream used for inoculation purposes since this seriously modifies the flavor and aroma of both the uninoculated and fermented milk.

---

[100] Ont. Agr. Col. Bul. 120. 1902.

Control of the bacterial flavors and aromas. The control of the bacterial flavors and aromas in milk and cream involves the prevention of the growth of the causative organisms. With small volumes of material the use of low holding temperatures is the most practical method while, with volumes that justify it, the use of pasteurization to destroy many of the objectionable organisms may logically precede the holding at satisfactory temperatures. It must be recognized that at all of the temperatures above the freezing point undesirable flavors and aromas will develop, the time of such development being largely dependent on the temperature and the numbers and types of organisms present. In many of the dairy plants great improvements have been made in the quality of the raw material by more rapid cooling on the farms and more frequent deliveries. Attempts to control the flavor and aroma fermentations in milk and cream should include more careful methods of production and handling so that contamination from such sources as utensils and the coats of the cows will be reduced to a minimum. The organisms gaining entrance will not cause abnormal flavors and aromas in milk or cream, however, unless growth occurs and this can best be controlled by low temperatures.

THE COLOR FERMENTATIONS

The action of micro-organisms may bring about changes in the color of milk and cream. Although such fermentations have occasionally caused trouble in Europe, they are of little practical importance in the United States and are unusual, even in milk and cream which have been allowed to stand for considerable periods.

There are many organisms commonly found in milk that produce colored colonies, especially yellow, orange or red, on agar and various other solid media but the development of color under these conditions is quite different than the color production in the typical color fermentations. Some of the organisms forming colored colonies show no color when grown in milk while others show only a colored sediment or surface ring in which there is a mass of organisms. In the typical color fermentations the color is distributed through considerable portions of the milk and is not confined to masses of growth. In general, the color changes involve the surface portion of milk and cream because of the oxygen requirements of the causative organisms. With the blue fermentation due to *Pseudomonas cyanogenes* and possibly with other color changes due to bacteria, associative action is important.

The blue fermentation. Although a number of organisms have been found responsible for a blue coloration in milk, the one most frequently isolated seems to be *Ps. cyanogenes.* The color produced, which is sometimes very intense, is formed only in the presence of acid.

A small outbreak[101] of blue milk was reported from an Iowa city by a dealer supplying 18 families. The color was observed by the consumers three times, twice in one household and once in another. The two families were related and kitchen utensils were frequently exchanged. The color was not observed on the producing farm, except in one instance when the container used was brought from one of the homes in which the blue color had been noted. The conditions on the farm were those commonly found and the organisms getting into the milk apparently came from the usual sources. In all instances the blue color was observed in sour milk and appeared in areas at the surface. The important characteristics indicating that the abnormality was due to an organism are, (1) it developed on standing, (2) it appeared in only a part of the milk supplied by the dealer and (3) the change was observed at the producing farm only in milk held in a container brought from one of the households experiencing the trouble. When a sample of the milk showing the blue color was studied in the laboratory the organism found to be most abundant was *S. lactis* but another type, which proved to be *Ps. cyanogenes,* was also present in considerable numbers. Neither type alone gave a blue color while a mixture of the two did. The history of the outbreak suggests that the original infection of the milk with *Ps. cyanogenes* occurred in one of the households having the trouble. After the organism was once established it was likely carried to the other house through the transfer of utensils.

Characteristics of Ps. cyanogenes. The striking characteristic of the blue fermentation of milk due to *Ps. cyanogenes* is that it does not occur except when there is acid formation. In pure culture in milk the organism fails to yield a blue color but a brownish tinge may develop. *Ps. cyanogenes* tends to produce an alkaline reaction in milk and it seems that, in combination with an acid producing organism, it may grow longer than it would alone, since the acid neutralizes the alkaline products formed. The organism yields a

[101] Ia. Agr. Expt. Sta. Res. Bul. 15. 1914.

material that presumably acts as an indicator and shows a definite blue color only in the presence of acid; however, the addition of acid to milk in which *Ps. cyanogenes* has grown alone does not result in the color change. The importance of acid in the color production is shown by the development of both acid and color in pure cultures of the organism in glucose bouillon. A blue fermentation may be produced in lactose bouillon by inoculating *S. lactis,* or some other acid producing organism, along with *Ps. cyanogenes.*

Blue milk due to non-bacterial causes. A blue color in milk may result from causes other than the growth of bacteria. A low content of solids and the eating of certain plants are among such causes; with these, the bluish tinge is evident as soon as the milk is drawn although it is more pronounced after the fat separates.

The yellow fermentation. A yellow color is sometimes produced in milk as a result of bacterial action and the most common organism seems to be *Pseudomonas synxantha.* This species has been found in the United States, but only incidentally, and apparently has not been noted under practical conditions as a cause of color changes; it has been isolated at the Iowa Agricultural Experiment Station twice, once from a bottle of milk that had been allowed to stand at room temperature until an intense yellow layer developed at the surface and once from a sample of milk drawn under careful conditions and held cold until it reached the laboratory.

With *Ps. synxantha*[102] the yellow color is pronounced in the cream layer and the typical color change seems to be associated with the fat. The organism proteolyzes casein and the digested material may have a yellow tinge, but a conspicuous color is found only in the fat layer. Sometimes black granules are also present in the fat and are undoubtedly made up of some decomposition product formed by the organism. *Ps. synxantha* produces a disagreeable odor in milk and the digested material may become viscous. The yellow color of butter can be intensified by growing *Ps. synxantha* in the cream from which it is made but the development of the color is accompanied by a very disagreeable flavor and aroma.

A number of organisms digest milk with the formation of a yellow color but these can not be regarded as of significance since the digestion usually requires considerable time and involves a pronounced change in the appearance and composition of the milk.

Yellow milk due to non-bacterial causes. The normal milk of certain animals, especially Guernseys, shows a yellow color which commonly varies in intensity with the season. Such feeds as carrots and mangels sometimes appear to cause a pronounced yellow color in milk. When there is an udder infection the milk may have a yellow tinge.

---

[102] Ia. Agr. Expt. Sta. Res. Bul. 20. 1915.

The red fermentation. A red color can be produced in milk by a number of organisms, such as *Serratia marcescens,* growing in pure culture; although these organisms are often found in milk and cream, the more rapid growth of other forms usually prevents their development.

Sour milk and cream, when held for considerable periods, may show red spots which are colonies of pink torulae; the most common of these is *Torula glutinis.* The pink torulae[103] are frequently present in milk and cream and are not restrained by the usual acidities developed; they produce red spots only occasionally, however, because of their comparatively slow growth. It is probable that in some instances the pink torulae get into dairy products from the air.

A red color in milk, as a result of the action of micro-organisms, must be distinguished from a red color due to the presence of blood. A ruptured vessel in the udder may result in blood getting into the milk and, if it is present in any considerable amount, the color of the milk will be changed. On standing a part of the erythrocytes settle to the bottom, while many are carried up with the cream, especially that making up the lower portion of the cream layer. Frequently blood is present in milk in such small amounts that the color is not perceptibly changed but such a condition can often be detected by centrifuging a small volume, when the erythrocytes are thrown into a distinct layer at the bottom of the centrifuge tube.

A red tinge in the glass has occasionally been the cause of a bottle of milk appearing to have a pink color; in these instances the cream especially appears pink since it provides a more definite background for the slight color in the glass.

THE VOLATILE ACID FERMENTATIONS

Various volatile acids may be produced in dairy products through the action of micro-organisms. Such compounds are especially important because of their pronounced influence on the aroma; in milk and cream they are always objectionable but in certain products, in which the action of organisms is necessary, they may be desirable.

[103] Jr. Dairy. Sc. 10. P. 210. 1927.

The Butyric Acid Fermentation

The butyric acid commonly produced through a fermentation process is the normal acid. It appears to be objectionable in dairy products under all conditions.

Importance of the butyric acid fermentation. The butyric acid fermentation is most frequently observed under practical conditions in sour cream in which there has been considerable bacterial action and is largely confined to the warm season. Butyric acid has a very objectionable rancid odor and its development in cream presents a real problem to some of the butter plants receiving their raw material from considerable distances. The use of a neutralizer partly removes the butyric acid odor from the cream through the formation of a butyrate. Under practical conditions the production of butyric acid ordinarily follows the development of considerable amounts of lactic acid.

General characters of the causative organisms. The important butyric acid organisms are anaerobic spore formers that produce gas and grow especially well at comparatively high temperatures. Most of them apparently tolerate considerable acid and this explains their growth in materials in which the lactic acid organisms have already been active. The essentially anaerobic conditions necessary for the growth of the butyric acid organisms are probably provided in milk and cream through the growth of various organisms, especially *S. lactis,* which use up the free oxygen. With at least some of these organisms there seems to be considerable protein decomposition which results in disagreeable odors other than those due to butyric acid.

Distribution of the organisms. Organisms capable of producing butyric acid are often present in milk and cream and the fermentation can ordinarily be initiated by making conditions favorable for their growth. This involves providing a comparatively high temperature and essentially anaerobic conditions, in addition to largely preventing the activity of other organisms. By heating a bottle, practically full of milk, to from 80° to 100° C. (176° to 212° F.) for a short time, to destroy the non-spore formers, and then cooling, corking tightly and holding at 37° C. (98.6° F.), the fermentation can often be started although, in some instances, the putrefactive anaerobes control the changes and produce very disagreeable odors.

The organisms capable of forming butyric acid get into milk, for the most part, either through direct fecal contamination or through contamination with soil which contains them because of the fecal material that has been added to it. The spore producing character enables the organisms to live for long periods of time, even under unfavorable conditions.

Clostridium butyricum. Although there are undoubtedly a considerable number of organisms that produce butyric acid in milk and cream, it seems that the common one is *Clostridium butyricum.* Some of the other butyric acid organisms are very closely related to it. The anaerobic character of these organisms makes their study rather difficult, especially from the standpoint of distinguishing differences.

The Propionic Acid Fermentation

Organisms of various types may form propionic acid in dairy products. This change is encountered in cream held for considerable periods and is undesirable there. In the ripening of Swiss cheese, the action of propionic acid organisms is very important because the gas evolved results in the development of the eyes. Propionic acid is also formed in small amounts during the ripening of butter cultures and cheeses other than Swiss, as a part of the desired change.

The Acetic Acid Fermentation

Different organisms are capable of producing acetic acid in dairy products. In old cream the odor of acetic acid may be pronounced and is then very objectionable. The changes occurring in certain fermentations, such as the ripening of butter cultures and various cheeses, commonly include the development of some acetic acid as a desirable part of the transformation.

NUMBERS OF MICRO-ORGANISMS REQUIRED TO PRODUCE DEFINITE CHANGES IN MILK

Milk may contain bacteria running into the millions per cubic centimeter without showing abnormalities of any kind and this suggests that, in general, considerable numbers must be present before off conditions develop. Hammer and Hix,[104] using the plate method, found that milk which showed changes of one kind or another always contained over 1,000,000 bacteria per cc. and ordinarily much larger numbers. The following table gives illustrative data from their results:

---

[104] Ia. Agr. Expt. Sta. Res. Bul. 29. 1916.

NUMBERS OF BACTERIA PRESENT IN MILK SHOWING VARIOUS CHANGES

Organisms	Milk Used	Condition Noted	Number of Determinations	Bacteria per CC.		
				Minimum	Maximum	Average
A. aerogenes	Sterile	Slightly ropy	7	11,000,000	49,000,000	32,200,000
A. aerogenes	Sterile	Very ropy	37	290,000,000	495,000,000	373,000,000
Bact. viscosum	Sterile	Slightly ropy	19	15,500,000	44,000,000	26,000,000
Bact. viscosum	Sterile	Very ropy	4	450,000,000	786,000,000	556,000,000
Bitter organism	Aseptic	Bitter flavor	10	10,000,000	66,000,000	38,900,000
Sweet curdler	Sterile	Slight coagulation	21	1,250,000	4,900,000	3,100,000
Sweet curdler	Sterile	Firm coagulation	26	15,950,000	56,000,000	28,500,000
Organism producing odor	Sterile	Faint odor	20	14,000,000	57,000,000	32,000,000
Organism producing odor	Sterile	Marked odor	37	66,000,000	150,000,000	95,000,000
Organism producing odor	Aseptic	Odor	31	19,000,000	98,000,000	48,000,000
S. lactis	Sterile	Slight reduction of litmus	15	62,000,000	181,000,000	117,000,000
S. lactis	Aseptic	Sour	4	53,000,000	166,500,000	104,000,000
S. lactis	Aseptic	Increased acidity but not distinctly sour	7	30,000,000	90,000,000	56,000,000

Among the organisms involved in these trials, the sweet curdler produced changes in milk with the smallest numbers of organisms, the minimum number observed with slight coagulation being 1,250,000 per cc. With the other organisms much larger numbers were required to produce definite changes. There were large differences between the minimum and maximum numbers noted for a given condition; these were undoubtedly due, in part, to the difficulty of securing uniformity in the extent of the condition in the various trials.

Determinations of the numbers of yeasts in cream showing various conditions, due to these organisms, on its arrival at butter plants have been made at the Iowa Agricultural Experiment Station, using the plate method and restraining the bacteria with tartaric acid; because of the gas in the foamy cream it was weighed out and the volume calculated on the basis of a specific gravity of one. The results obtained are as follows:

Yeasts in Cream Showing Abnormal Conditions

Condition of Cream	Yeasts per CC.
Yeasty odor	1,585,000
Yeasty odor	2,150,000
Decidedly yeasty odor	10,550,000
Decidedly yeasty odor	12,800,000
Foamy	6,250,000
Foamy	11,150,000

These counts suggest that, with the yeasts involved, smaller numbers are required to produce extensive changes than with most of the bacteria; the comparatively large size of the yeast cells may be a factor in this.

CHARACTERISTICS OF MILK ABNORMALITIES DUE TO THE GROWTH OF MICRO-ORGANISMS

Abnormal conditions due to the growth of micro-organisms have certain characteristics that ordinarily render them distinguishable from abnormalities due to other causes and, frequently, a consideration of the general nature of an unusual condition makes it possible to determine whether or not micro-organisms are responsible. The more important of these characteristics are the following:

1. The abnormal condition is usually not present when the milk is drawn. A change in milk, such as ropiness or an abnormal color, that is due to the activity of micro-organisms would not be expected until there was an opportunity for growth, while resembling abnormalities due to the condition of the animal should be present when the milk is first drawn. A flavor and aroma due to the action of micro-organisms would not be present at the time of milking, while one due to the feed of the animal would. When using this character in connection with flavors and aromas, the possibility of absorption from the surroundings must be taken into account.
2. The abnormal condition usually increases on standing. In general the increased growth of the organisms results in an increase in the extent of the abnormal condition due to them, as would be expected. Ropiness, for example, becomes more pronounced with an increase in the time of holding, if the temperature remains favorable for the growth of the organisms. Eventually there is likely to be a disappearance of any abnormal condition, whether due to micro-organisms or other causes, because of the extensive growth of various organisms.
3. In many instances the abnormal condition can be inoculated from one lot of milk to another. The organisms causing an abnormality in milk will often produce the same condition when a bit of the milk is transferred to a fresh lot, preferably sterile. There are, however, a number of factors, such as the growth of organisms other than the causative type and the use of unsatisfactory temperatures, that may be responsible for failures in such trials. When the reproduction of the abnormal condition is to be attempted, it is much better to work with cultures, either pure or in known mixtures, than with the abnormal material direct.

Important exceptions. The rancid, bitter or salty flavor in the milk of animals advanced in lactation and the tallowy flavor and aroma due to sunlight show certain characters suggesting that they are caused by micro-organisms; for example, they increase on standing and are commonly not present when the milk is first drawn. These flavors are so characteristic, however, that they are readily recognized and the cause assigned. Bottled milk sometimes acquires a flavor from the cap under conditions suggesting the action of organisms.

CHAPTER III

CONTAMINATION OF MILK AND CREAM

During the production and handling of milk, micro-organisms are ordinarily added to it from a variety of sources. The numbers present at the time milk leaves the stable vary from a few thousand, or even less than one thousand with very careful methods, to half a million or more when unsatisfactory conditions of production prevail. In the various plants handling milk and in the homes in which it is consumed, the addition of organisms commonly continues, although with proper care this contamination may be negligible. The temperature and time of holding largely determine the extent of the growth that occurs but the contamination provides the beginning of this development and thus assumes an importance beyond the actual numbers of organisms added.

Because of differences in the general character and importance of the organisms involved, the contamination of milk may logically be divided into the following types:

1. Contamination from the interior of the udder.
2. Contamination from external sources.

CONTAMINATION FROM THE INTERIOR OF THE UDDER

At one time it was rather commonly believed that milk, especially that drawn toward the close of a milking, could be secured in a sterile condition. This idea was not the result of careful investigations and seems to have been largely based on trials in which a few cubic centimeters of apparently sterile milk were secured and on the fact that milk obtained aseptically often shows but little tendency to undergo rapid or conspicuous changes. Both Moore[1] and Ward[2 and 3] have obtained results which make this idea untenable; they examined the udders of cows killed after being milked dry and found that organisms could be regularly secured from the glandular tissue. The types recovered seemed to be identical with certain of those found in carefully drawn milk. Ward reported that the lactiferous ducts of the 19 udders examined, harbored bacteria throughout their whole extent and concluded that, "Milk, when secreted by the glands of the healthy udder, is sterile. It may, however, immediately become contaminated by the bacteria which are normally present in the smaller milk ducts of the udder."

---

[1] Proc. Soc. Prom. Agr. Sc. P. 110. 1899.

[2] Jr. App. Mic. 1. P. 205. 1898.

[3] N. Y. (Cornell) Agr. Expt. Sta. Bul. 178. 1900.

It is now generally agreed that milk contains bacteria at the time it is drawn, as a result of contamination in the milk cistern and the ducts leading into it. The growth of organisms in these passages during the periods between milkings provides the supply that makes possible the continued contamination of the milk.

Numbers of bacteria in milk drawn aseptically. The organisms present in milk drawn aseptically are difficult of accurate enumeration; the tendency to form clumps suggests unusually large errors with the plate method and the numbers are ordinarily too small for the most satisfactory microscopic counts. The plate method has been used, for the most part, in the examinations reported. There is considerable variation in the results obtained by different investigators. Some of the early studies yielded higher counts than the more recent ones, due, in part at least, to less careful methods of securing the samples.

Harding and Wilson [4] have reported extensive results secured on milk drawn under careful conditions from the udders of 78 cows, the samples commonly being obtained at the close of the milking. The data on the numbers of organisms present may be summarized as follows:

BACTERIAL CONTENT OF ASEPTICALLY DRAWN MILK

Source of Samples	Number Samples	Average Number Bacteria per CC.
N. Y. (Geneva) Agr. Expt. Sta. Herd, 1900–1902..........	316	518
N. Y. (Geneva) Agr. Expt. Sta. Herd, 1910–1911..........	730	420
Farm Herd, 1910–1911..................................	184	320
Total..	1,230	428

Only 8 per cent of the 1,230 samples examined contained over 1,000 bacteria per cc.

---

[4] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 27. 1913.

Copeland and Olson[5] determined the numbers of bacteria in milk from each quarter of the udders of 40 cows, the samples being taken after approximately one-third of the milk had been drawn from the quarters. Omitting unusually high counts secured with one animal, the average number was 1,541 per cc. Breed,[6] using a dilution of 1 to 10 in pouring the plates, found an average bacterial content of 964 per cc. for 45 samples of strippings milk drawn directly into sterile test tubes from individual quarters; in 8 instances the counts were between 1,000 and 15,000 per cc.

There is commonly considerable variation in the counts obtained on milk from the different animals and this suggests that milk having a count decidedly lower than the averages given might be secured. Such milk has been reported from various sources. The carefully prepared samples submitted to milk scoring contests have shown many very low counts, sometimes under 100 per cc. Atwood and Giddings[7] found an average of 35 bacteria per cc. and extremes of 6 and 120 for six samples of approximately two quarts each that came from one cow; only middle milk from the rear quarters was included.

Excessive counts on milk drawn aseptically. Occasionally, animals with apparently normal udders are found giving milk that regularly contains unusually large numbers of bacteria. Two such cows were studied by Hastings and Hoffmann[8] and the results, together with data secured on a third animal giving milk containing normal numbers of bacteria, are as follows:

EXCESSIVE BACTERIAL COUNTS ON MILK DRAWN ASEPTICALLY

Cow	Number of Samples	Bacteria per CC.		
		Average	Minimum	Maximum
Brownie	61, covering 15 mo. and parts of two lactation periods	30,700	1,700	305,000
Dorine	31, excluding 2 very high counts	38,800	2,500	154,000
Dorine	33	191,000*	2,500	3,500,000
Merney	27	800	50	4,250

* See ref. 4.

---

[5] S. D. Agr. Expt. Sta. Bul. 218. 1926.

[6] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 132. 1928.

[7] W. Va. Agr. Expt. Sta. Bul. 134. 1911.

[8] Wis. Agr. Expt. Sta. Res. Bul. 6. 1909.

Copeland and Olson[9] made numerous counts, during one lactation period, on the milk of a cow with an apparently normal udder and found that 40 exceeded 50,000 per cc. and 17 exceeded 100,000; the highest count obtained was 347,000 per cc.

Cows giving milk containing excessive numbers of bacteria are undoubtedly of significance in the production of milk that must comply with rigid bacterial standards. In certain instances the failure of certified supplies to come up to the bacteriological requirements has been due to a few cows giving exceptionally high count milk, and the exclusion of this milk has quickly brought the numbers of organisms within the limits set. Such cows seem to have normal udders and cannot be detected except by running counts on milk from the individual animals in the herd.

Distribution of organisms throughout the milking. There is considerable variation in the numbers of organisms per cubic centimeter in different portions of the milking, the fore milk quite regularly containing the largest number. In a series of comparisons von Freudenreich[10] found the fore milk averaged 6,505 per cc., the middle milk 1,341 and the strippings 769; the fore milk did not always show the highest count in these comparisons. The following table, taken from results secured by Stocking,[11] illustrates how the number of organisms decreases as the milking proceeds:

Bacterial Content of Various Portions of a Milking

Number of Trial	Bacteria per CC. in				
	Streams 1 and 2	Streams 5 and 6	Streams 9 and 10	Streams 13 and 14	Strippings
1	1,940	550	250	275	216
2	25,200	5,391	285	218	101
3	5,491	2,096	430	820	144
4	7,941	1,350	125	216	156

Harding and Wilson[12] examined the milk from five cows on six successive days, the milk from each quarter being studied separately, and found the general averages to be as follows: fore milk 458 per cc., middle milk 187 and last milk 274. In attempting to determine the accuracy of a count on the strippings, as a measure of the bacterial content of the whole milking, these investigators examined the strippings and the whole milking minus the fore milk from a cow on three successive milkings. The cow was carefully groomed, her flanks and udder were moistened and her teats and the hands of the milker were vaselined. A half-pint of milk was rejected from each quarter and the remainder drawn into a sterile bottle. The average of the strippings was 365 per cc. and of the whole milk 305. Orla-Jensen[13] secured milk in sterile bottles after washing the udder and teats and found that the fore milk from four quarters contained 16,000 bacteria per cc., the middle milk 480 and the strippings 360.

---

[9] See ref. 5.

[10] Centbl. f. Bakt. 2 Abt. 13. P. 281. 1904.

[11] Conn. (Storrs) Agr. Expt. Sta. 18th An. Rpt. P. 66. 1906.

[12] See ref. 4.

Copeland and Olson[14] determined the numbers of bacteria in the fore milk, middle milk and strippings from each quarter of eight cows. Omitting the counts from one infected quarter, the fore milk averaged 5,989 per cc., the middle milk 557 and the strippings 415. The following data illustrate the general trend of their results:

Bacteria in Fore Milk, Middle Milk and Strippings

	Bacteria per CC.		
	Fore Milk	Middle Milk	Strippings
Right front quarter	1,110	71	21
Left front quarter	1,600	51	18
Right rear quarter	2,710	101	13
Left rear quarter	1,760	349	117

The comparatively high bacterial content of the fore milk is undoubtedly due to this portion of the milk washing out most of the easily removable organisms present in the milk cistern and teat canal. Contamination from these sources and the milk ducts persists during the remainder of the milking through the gradual dislodging of organisms but is less per cubic centimeter of milk. The manipulation of the udder incident to the stripping of an animal apparently may result in an increased dislodging of organisms and thus cause a larger number of bacteria in the strippings than in the middle milk.

---

[13] Orla-Jensen, Dairy Bacteriology. P. 62. 1921.

[14] See ref. 5.

Numbers of bacteria in milk from different quarters. The results secured by various investigators show wide variations in the bacterial content of milk from different quarters of an udder. This is illustrated by the following representative data taken from the work of Copeland and Olson:[15]

Bacterial Content of Milk from Different Quarters

	Bacteria per CC. in Milk from				
	Cow 3	Cow 5	Cow 7	Cow 9	Cow 14
Right front quarter	5,080	7	2,165	285	585
Left front quarter	9	180	47	1,320	1
Right rear quarter	450	9,450	780	14	5
Left rear quarter	2	28	540	4,850	4

von Freudenreich and Thöni[16] have also reported differences in the types of organisms from different quarters.

Atwood and Giddings[17] found the bacterial content of the milk from the front quarters to be higher per cubic centimeter than that from the hind quarters; these investigators pointed out that, with such a distribution, the quarters producing the larger amount of milk have a smaller bacterial count per cubic centimeter. Harding and Wilson,[18] however, reported about three times as many bacteria per cubic centimeter in the milk from the hind quarters as in that from the front quarters and stated that, "The factor controlling this distribution has not been found."

Relation of the period of lactation to the number of bacteria in milk. Atwood and Giddings[19] made a number of examinations of colostrum milk from a heifer and found the counts varying from 35 to 3,350 per cc. Harding and Wilson[20] reported data in which the period of lactation had no marked influence on the bacterial content of the milk, although the colostrum milk had a slightly higher count than that secured later. Copeland and Olson[21] determined the numbers of organisms in the colostrum milk from each quarter of six cows, the samples being collected within six hours of calving and in three cases before the calf had suckled. Most of the counts were rather high but a number were under 1,000 per cc.; the maximum was 650,000 per cc. There were the usual variations in the milk from different quarters. These investigators studied four cows for 14 months, making counts every five days, and found that the average monthly bacterial counts tended to remain fairly constant; the results also showed that variations in the bacterial counts of the milk from the different quarters may be permanent.

---

[15] See ref. 5.

[16] Rev. Gén. du Lait. 2. P. 241. 1903.

[17] See ref. 7.

[18] See ref. 4.

[19] See ref. 7.

[20] See ref. 4.

[21] See ref. 5.

Influence of the age of the animal on the number of bacteria in milk. Harding and Wilson [22] studied the possible influence of the age of the animal on the bacterial content of milk by tabulating the results obtained on 1,085 samples according to the ages of the producing cows; although there was considerable variation in the average counts for the different groups, no distinct age influence was evident. Copeland and Olson [23] divided the animals used in their work into age groups and averaged the bacterial counts on the milk of each; the average for the animals over seven years of age was higher than the averages for the younger groups. It is, of course, possible that the greater average number of udder infections with the animals that had been milking longer may have resulted in a lessened resistance of the udder tissues so that more organisms were present.

Influence of the type of feed on the bacterial content of milk. The character of the feed consumed does not seem to bear any relationship to the bacterial content of milk drawn aseptically. This would be expected since organisms are not ordinarily carried from the intestinal tract to the udder. The character of the feed may, however, markedly influence the consistency of the fecal material and, in this way, the contamination from such external sources as the coat of the cow.

Influence of incomplete milking on the bacterial content of the next milking. The data reported by Stocking [24] indicate that, when an animal is not milked dry, the milk drawn at the following milking has a higher bacterial content per cubic centimeter than when the animal is milked dry. Ten comparisons were made on one cow and the average bacterial content of the milk drawn at the milkings following incomplete milking was 11,324 per cc., while the average for that drawn at the milkings following complete milking was 6,542. Stocking suggests that, without the usual manipulation incident to complete

[22] See ref. 4.

[23] See ref. 5.

[24] See ref. 11.

stripping, more organisms are left in the udder and the growth of these makes possible a heavier contamination of the milk at the succeeding milking than if smaller numbers had been left.

Types of organisms in milk drawn aseptically. The organisms present in milk drawn aseptically belong to certain more or less definite types and are not of a wide variety of species. To multiply in the udder, an organism must have the ability to grow in contact with the tissues there, and this is a character lacking in many common types. Some of the species that develop rapidly in milk after it is drawn, die out quickly when introduced into the udder. Ward [25] injected *Serratia marcescens* through the teat canal and, although an infection was set up, found that only a few colonies developed on plates poured after 24 hours while none developed on plates poured on the sixth day. Russell and Hastings [26] reported results on the injection into the udder of four species, the common lactic organism, an acid producing liquefying form, *S. marcescens* and a yellow liquefying coccus frequently found in fore milk; in all cases the numbers of these organisms recovered in the plates became less and less as the period of exposure in the udder increased. In most instances the injection of the organisms resulted in the milk becoming more or less abnormal and sometimes the udder was inflamed. Copeland and Olson [27] injected *Aerobacter aerogenes* into one quarter and, although this became swollen and enlarged, the organism did not survive more than a few days.

Micrococci are particularly prominent among the organisms isolated from aseptically drawn or very low count milk and are also conspicuous among the organisms secured from the udder tissues. Evans [28] studied 192 samples of milk from 161 cows, representing five different dairies, and found micrococci in 113 or 58.9 per cent; the highest number was 80,000 per cc. Many of these micrococci belonged to one group, which agreed in its characteristics with the pyogenic staphylococci, but the most of the cultures of this type were non-virulent; two cultures, however, possessed such a high virulence that inoculated rabbits died in 16 hours. Three types of micrococci gave reactions which differed from the pyogenic staphylococci; one was identified as *M. luteus,* one was characterized by a rapid and complete peptonization of milk and was named *M. caseolyticus* while the third was not named.

---

[25] See ref. 2.

[26] Wis. Agr. Expt. Sta. 21st An. Rpt. P. 164. 1904.

[27] See ref. 5.

[28] Jr. Inf. Dis. 18. P. 437. 1916.

Breed[29] identified 171 of 176 cultures of micrococci isolated from carefully drawn strippings milk, with the following results: *M. aureus,* 33 cultures; *M. aurantiacus,* 24 cultures; *M. freudenreichii,* 23 cultures; *M. albus,* 21 cultures; *M. candidus,* 20 cultures; *M. epidermidis,* 13 cultures; *M. citreus,* 10 cultures; *M. varians,* 10 cultures; *M. flavus,* 8 cultures; *M. conglomeratus,* 4 cultures; *M. luteus,* 3 cultures; *M. casei,* 2 cultures. The remaining five cultures gave a red or pink growth.

Although the udder micrococci show considerable variation they agree in producing changes in milk comparatively slowly and thus are of little importance in the deterioration of milk under practical conditions.

Streptococci are also present in milk from normal udders. Sherman and Hastings[30] found them in 0.01 cc. of the mixed milk from 10 out of 12 herds and in 38.6 per cent of the individual samples from 88 cows in 4 herds. Evans[31] secured long chained streptococci, which were not *S. lactis,* in 29 or 15.1 per cent of the 192 samples studied, the highest number being 264,000 per cc.

By means of special media, especially agar to which blood serum has been added, rod shaped bacteria may often be isolated from milk drawn aseptically. Evans[32] recovered them from 45 or 23.4 per cent of the 192 samples of milk examined, the maximum number being 50,000 per cc. In another series of 23 individual samples, this investigator[33] found that 17 or 73.9 per cent contained the rod forms and the maximum number was 112,000 per cc.; if the 23 samples of milk had been mixed, there would have been 7,000 of these organisms per cc. The organisms grow rapidly in milk; they are of different types and one is of special interest since it hydrolyzes butter fat and produces a disagreeable flavor and odor in cream within 24 hours, if the initial contamination is high.

Unusual organisms in milk drawn aseptically. It appears that occasionally organisms other than the usual udder types are present in milk as a result of contamination in the udder; for example, Moore and Ward[34] investigated the development of gas and taints in the curd in a cheese factory and found the cause to be an organism belonging to the Escherichia-Aerobacter group which apparently was present in the milk of certain animals at the time it left the udder. When organisms capable of producing rapid changes are present in the udder, the deterioration of the milk or the products for which it is used may be very rapid.

---

[29] See ref. 6.

[30] Creamery and Milk Plant Monthly. 3. P. 11. Feb. 1915.

[31] See ref. 28.

[32] See ref. 28.

[33] Jr. Bact. 2. P. 185. 1917.

[34] N. Y. (Cornell) Agr. Expt. Sta. Bul. 158. 1899.

Pathogenic organisms in milk drawn aseptically. As a result of an infection in the producing animal, milk sometimes contains pathogenic organisms at the time it leaves the udder. The organisms causing tuberculosis, contagious abortion and certain udder infections are the most important of these in cows' milk, while the organism causing Malta fever is the most serious in the case of goats' milk. The presence of these organisms in milk and their general importance are discussed in Chapter VII.

CONTAMINATION FROM EXTERNAL SOURCES

The contamination of milk from external sources may conveniently be divided into (1) that taking place on the producing farms and (2) that occurring subsequent to production and involving especially the contamination from the equipment in the plants handling milk.

Contamination on the Producing Farms

On the producing farms the sources from which micro-organisms may be added to milk are (1) the stable air, (2) the utensils, (3) the coat of the animal, (4) the milker, and (5) miscellaneous sources.

(1) The Stable Air

The air of stables frequently contains dust in considerable quantities and this has led to the assumption that the air is an important factor in the contamination of milk. Investigations have shown, however, that although some organisms get into milk from this source, the numbers are ordinarily not as large as might be expected; it seems probable that the desiccation and light, to which the organisms carried by the dust are subjected, destroy many of them so that the material falling into milk from the air adds comparatively few organisms in a living condition.

Methods of measuring contamination from the air. The extent of the contamination of milk from the air has been investigated in various ways. One of the simplest methods used has been to expose agar plates of a known area for a given length of time in the stable to be studied and then calculate, from the number of colonies developing on incubation, the organisms that would fall into a pail with a certain opening during the milking period. This method is not at all satisfactory since the organisms present on a dust particle may be so close together that several develop into a single colony, instead of one colony being formed for each organism, and it is also probable that some organisms are held away from the surface of the agar and do not develop at all. Sterile milk or water may be exposed in a pail and the organisms falling in from the air determined by making counts. Variations in the air conditions may be shown by counting the organisms per liter of air by means of aeroscopes but such data give no direct information on the numbers of organisms added to milk. Ruehle and Kulp[35] have devised an "artificial udder" from which sterile water can be "milked"; the bacterial content of this water is then determined.

The effect on the bacterial content of the milk of increasing the amount of dust in the air by some such process as feeding may be investigated by milking half of a group of cows when the air is comparatively free from dust and the other half after carrying out the operation being studied; samples for the counts are taken as soon as the milk is drawn. The two lots of cows are ordinarily alternated in different comparisons to compensate, as far as possible, for the variations in the contamination from the interiors of the udders.

Effect of feeding just before milking. Stocking[36] studied the effect of feeding hay and grain, just before milking, on the bacterial content of milk, using 10 cows in six comparisons and determining the organisms by the plate method. The results obtained are as follows:

INFLUENCE OF FEEDING HAY AND GRAIN JUST BEFORE MILKING ON THE BACTERIAL CONTENT OF MILK

	Bacteria per CC.		
	Total	Acid Producing	Liquefying
Before feeding	2,096	790	108
After feeding hay and grain	3,506	1,320	196
Increase caused by feeding	1,410	530	88

---

[35] N. Y. (Geneva) Agr. Expt. Sta. Bul. 409. 1915.

[36] See ref. 11.

The effect of feeding dry corn stover was studied by the same investigator, using two cows in five comparisons, with the following results:

INFLUENCE OF FEEDING CORN STOVER JUST BEFORE MILKING ON THE BACTERIAL CONTENT OF MILK

	Bacteria per CC.			Molds per CC.
	Total	Acid Producing	Liquefying	
Before feeding	1,233	297	1	118
After feeding dry corn stover	3,656	692	28	123
Increase caused by feeding	2,423	395	27	5

Both of the comparisons show a high percentage increase in the numbers of organisms as a result of handling dusty materials in the stable just before milking. The actual increases are small, when compared to the contamination from certain other sources, but would be important in the production of low count milk and especially objectionable since they can so easily be prevented.

Effect of brushing animals just before milking. The effect of the dust raised by brushing the cows, just before milking, on the bacterial content of milk was studied by Stocking[37] using 10 cows in 14 comparisons; the results obtained are shown in the following summary:

INFLUENCE OF BRUSHING COWS JUST BEFORE MILKING ON THE BACTERIAL CONTENT OF MILK

	Bacteria per CC.		
	Total	Acid Producing	Liquefying
Not brushed	1,207	213	59
Brushed just before milking	2,286	381	117
Increase caused by brushing	1,079	168	58

---

[37] See ref. 11.

The percentage increase in the number of organisms due to brushing just before milking was large but the actual increase rather small; if rigid bacterial standards were being met, the actual increase would be of considerable importance.

Bacterial content of stable air. Ruehle and Kulp[38] have investigated the bacterial content of stable air and its effect upon the bacterial content of milk, using aeroscopes and the ''artificial udder'' from which sterile water could be ''milked.'' When the cows were in the stable the germ content of the air was usually between 50 and 100 per liter, with a maximum of 825. Sterile water ''milked'' under these conditions had an average bacterial content of 12 per cc., the highest count being 73. When a heavy dust was raised in the stable loft by sweeping up material from the floor the bacterial content of the air was from 1,000 to 2,000 per liter and sterile water ''milked'' under these conditions had an average bacterial content of 47.6 per cc. By raising dust continuously during the test the bacterial content of the air was increased to between 2,500 and 10,000 per liter and the count of the water ''milked'' averaged 604 per cc. Fifty-eight analyses of the air in three commercial laboratories gave results quite in agreement with those secured in the stable investigated, except in four instances in which the results were like those secured in the dusty loft. On exposing a sterile fluid to the air of a stable for five minutes, the counts indicated an average of 55 organisms for each square centimeter of surface when chopped hay was being fed, 71 when dry grain was being fed, and 114 when milking was in progress. Under these conditions, if five liters of milk had been exposed in an open 12 inch pail for one hour, the organisms added to the milk would have been 99, 124 and 199 per cc. respectively. On raising a heavy dust in the stable loft the average number of organisms per square centimeter, after a five minute exposure, was 678 when the dust was raised but once and 28,377 when the dust was stirred up continuously. The bacterial content of a pail of milk exposed to such heavily contaminated air would be increased from one to several thousand bacteria per cc. in a few minutes' exposure.

Types of organisms from air. Because of the severe conditions existing in the air, the organisms coming from this source must be, for the most part, those that are comparatively resistant. They include the spore forming bacteria, both aerobic and anaerobic, certain cocci, especially micrococci, and various types of yeasts and molds.

---

[38] See ref. 35.

Organisms that have very little resistance are sometimes encountered; presumably they have been in the air for only a short time.

(2) The Utensils

Unless the utensils with which milk comes in contact are steamed, scalded or treated in some other way which largely destroys the organisms in them, they are quite likely to be serious sources of contamination. On the ordinary dairy farm, where little attention is given to the utensils, they are often the most important source of the bacteria in milk from the standpoint of numbers.

Methods of measuring contamination from utensils. A common method of showing the contamination from a utensil is to add a known volume of sterile water, get the organisms suspended in this as completely as possible by shaking or brushing with a sterile brush, determine the bacteria per cubic centimeter of the water and then calculate the number in the utensil. This procedure may be modified by using milk and determining the bacteria per cubic centimeter before and after it is put into the utensil, care being taken to keep the time involved so short that there will be no opportunity for growth. Either method gives results that are somewhat low because of the difficulty of quickly dislodging all of the organisms from the utensil.

Numbers of organisms coming from utensils. Prucha, Weeter and Chambers[39] found that 170 freshly washed and unsteamed cans contained bacteria sufficient to have added from 197 to 2,557,000 organisms per cc. with an average of 128,592, if the cans had been filled with milk. An investigation of 91 cans that were washed, rinsed and steamed at the plant and then covered with their lids and returned to the farms showed that, as they were about to be used, they would have added 23,523 bacteria per cc. to the milk; the treatment on the farms was not uniform, either as to the cans being kept covered or as to the time of holding. The examination of 154 bottles that were freshly washed, but unsteamed, indicated that they would have added 1,339 bacteria per cc., had they been filled with milk, while 154 that were washed and unsteamed and then left inverted on a rack for 24 hours would have added 12,930 per cc. The collective influence of the utensils was studied on 81 cans of milk drawn and handled with sterile utensils in comparison with 117 cans for which the utensils were not sterilized; the average bacterial content of the former was 6,807 per cc. and that of the latter 285,600.

---

[39] Ill. Agr. Expt. Sta. Bul. 204. 1918.

North[40] has strikingly shown the effect of unsterile cans on the bacterial content of milk by the following data secured on a shipment of 4,000 gallons:

INFLUENCE OF UNSTERILE CANS ON THE BACTERIAL CONTENT OF MILK

	Bacteria per CC.
Milk from 60 farms at shipping station	5,000,000
Milk pasteurized	6,700
Milk 3 minutes after adding to unsterile 10 gallon cans	560,000
Milk in New York City the following day	12,000,000

Smith[41] found in the examination of 236 cans under all conditions of washing and steaming that, had the cans been filled with milk, the contamination would have varied from 0 to 7,920,000 organisms per cc. of milk. A study of 268 bottles, most of which were pints, showed that from 0 to 1,636 bacteria would have been added per cc. of milk. Smith[42] later reported additional data. Cans were examined at the railway station as they were being sent to shippers; 108 wet cans would have added 547,994 bacteria per cc. of milk with extremes of 52 and 4,332,000, while 38 dry cans would have added 1,870 bacteria per cc. with variations from 2 to 19,632. Sixty cans from four plants using washing machines and air blasts for washing and drying the cans, would have added 14 bacteria per cc. of milk with extremes of 0 and 331. In one plant the effect of improved washing equipment on the condition of the cans was clearly evident; with the old method the 9 cans studied would have added 88,500 bacteria per cc. of milk, with variations from 23,700 to 178,000, while after a can washing machine with an attached air blast was installed 12 cans would have added 122 bacteria per cc. with variations from 52 to 634. A total of 344 bottles from 32 plants were studied and the average contamination of milk under all conditions of washing would have equalled 288.3 organisms per cc.; without a mechanical washer and steamer the contamination from 190 bottles would have averaged 458.2 per cc. with a range from less than 1 to 9,005, while with a mechanical washer and steamer it would have averaged 78.8 per cc. from 154 bottles with a range from less than 1 to 900.

[40] Am. Jr. Pub. Health. 7. P. 25. 1917.

[41] Jr. Dairy Sc. 2. P. 487. 1919.

[42] Jr. Dairy Sc. 3. P. 540. 1920.

Growth of organisms in utensils. Although wash water may contain millions of organisms per cubic centimeter because of the contamination from the utensils washed in it, this can not account for the enormous numbers often present in pails, cans, etc. and growth must be an important factor. The extent of the growth is determined by the conditions existing and the types of organisms involved. Water containing traces of milk solids provides a suitable medium for many organisms, while the temperatures at which utensils are ordinarily held, especially during the warm months, are satisfactory for development. When the washing is carelessly done so that milk solids remain on the utensils in considerable amounts, the number of organisms left after the washing is likely to be high and, in addition, growth is greatly favored by the nutrients thus provided. Rough areas and sharp depressions on the inner surface of a utensil make the complete removal of the milk solids difficult and thus complicate the washing. The growth of organisms in utensils is also objectionable because of the odors developed and because some of the products formed accelerate the action on the metal.

Strainers and separators. The contamination of milk from strainers and separators may be just as important as that coming from utensils. Metal strainers are ordinarily washed with the cans and pails and are, accordingly, subjected to the same general conditions. If a strainer cloth is used, it is sometimes left moist so that there is a good opportunity for bacterial growth.

Separators are not always washed every time they are used and the milk and separator slime left in them afford an ideal food supply for bacteria so that, at suitable temperatures, rapid growth occurs; when the machines are then used, the addition of large numbers of organisms to the skim milk and cream is to be expected. The growth of organisms in unwashed separators, especially during the warm season, frequently results in very undesirable odors which are taken up by the material run through the machines. The experienced cream judge often detects objectionable flavors and aromas that are recognized as coming from unclean separators.

Types of organisms from utensils. The types of organisms added to milk as a result of contamination from the utensils are likely to be rather varied. It is to be expected, however, that the organisms that grow well in milk would be prominent among them, since the conditions for growth in the utensils are similar to the conditions in milk. This suggests that *S. lactis* would frequently be added from the utensils and, under average farm conditions, pails and cans are perhaps the most important source of this organism. In certain cases ropy milk organisms have come from the utensils and this must always be taken into consideration in attempting to control outbreaks of ropiness. Organisms which do not grow especially well in milk may remain more or less inactive in the utensils and then be added to the milk when the utensils are used. In attempts to reduce bacterial counts or control various fermentations the possibility of the infection coming from the utensils must always be taken into account.

Contamination from milking machines. The introduction of milking machines has provided an additional source of bacteria on the producing farms that is very serious, unless proper methods of treatment are employed. The early investigations of the bacteriological condition of machine drawn milk indicated that the counts were commonly higher than those of hand drawn milk. Harrison,[43] who carried out some of the very early studies, found that 235 samples of machine drawn milk contained from 71,124 to 243,327 bacteria per cc. with most of the counts between 100,000 and 200,000, while 94 samples of hand drawn milk varied from 645 to 63,422 per cc., with the numbers usually between 1,000 and 10,000; these results are illustrative of many that have been secured in both Europe and America.

Hastings and Hoffmann[44] reported results as follows: of 150 samples of machine drawn milk, 22 per cent contained less than 1,000 per cc., 55.3 per cent contained between 1,000 and 5,000 and 22.7 per cent contained more than 5,000, while of 136 samples of hand drawn milk, 19.8 per cent contained less than 1,000 per cc., 44.1 per cent contained between 1,000 and 5,000 and 36.1 per cent contained more than 5,000. Edwards[45] found counts from 203,000 to 1,208,000 per cc. with seven samples of machine drawn milk, while six counts on hand drawn milk ranged from 3,200 to 68,900; when the rubber parts and cover of the machine were boiled once a week the count was somewhat reduced and, with special precautions in the preparation of the machine, two samples were secured with counts of 1,407 and 1,776 per cc. Meek[46] noted averages for a considerable number of samples as follows: milk from individual cows, hand drawn 21,115 per cc.; milk from individual cows, machine drawn 47,870 per cc.; mixed milk, hand drawn 26,895 per cc.; mixed milk, machine drawn

[43] Ont. Agr. Col. 23rd An. Rpt. P. 128. 1897.

[44] Wis. Agr. Expt. Sta. 24th An. Rpt. P. 214. 1907.

[45] Ont. Agr. Col. Bul. 159. 1907.

[46] Pa. Agr. Expt. Sta. An. Rpt. 1907–8. P. 146. 1908.

83,143 per cc. Harding, Wilson and Smith [47] found that 20 samples of machine drawn milk averaged 692,542 per cc., while an equal number of hand drawn samples averaged 16,643; the cows supplying the samples were milked alternately by hand and machine. Larsen, White and Fuller,[48] in 38 comparisons, noted that the bacterial content of the hand drawn milk averaged 10,739 per cc. while that of the machine drawn milk averaged 509,285.

Sources of the bacteria in machine drawn milk. It was early assumed that the high bacterial counts so often secured on machine drawn milk were due to contamination from the machine itself and this was readily proved by the reduction in the counts obtained when the machines were given careful attention. The more or less complicated construction makes cleaning difficult on the ordinary farm unless special methods are used and carelessness appears to be quickly reflected in the bacterial content of the milk.

Ruehle, Breed and Smith [49] found that the chief source of bacteria, when large numbers were added to milk by a milking machine, was the teat cups and rubber tubes, although the pails were also important in some cases. By a special device permitting sampling, these investigators compared the contamination from various parts of the machine when sterile water from an "artificial udder" was drawn through it. The summary of 13 tests of 4 milkings each, with which the counts were determined by the plate method, is as follows:

CONTAMINATION FROM VARIOUS PARTS OF A MILKING MACHINE
(13 TRIALS)

	Bacteria per CC. from		
	Teat Cups Only	Teat Cups and Head	Entire Machine
First milking	564	4,144	8,473
Second milking	686	2,065	3,038
Third milking	248 *	6,008 *	3,606 *
Fourth milking	752 *	421 †	2,831 *

* Average of 12 trials. † Average of 9 trials.

---

[47] N. Y. (Geneva) Agr. Expt. Sta. Bul. 317. 1909.

[48] S. D. Agr. Expt. Sta. Bul. 144. 1913.

[49] N. Y. (Geneva) Agr. Expt. Sta. Bul. 450. 1918.

Air contamination was unimportant, as would be expected from the information available on the bacterial content of stable air. Dropping the teat cups on the floor caused relatively large amounts of dirt to appear in the pails but analyses of sterile water drawn through the machines at the same time did not show the high bacterial counts that might be expected, since these conditions failed to give values in excess of 25,000 per cc.

The importance of condensation water from the vacuum line as a source of the bacteria in machine drawn milk is evident from the studies of Stocking and Mason[50] and of Moak.[51] The investigations of Breed and Bright,[52] reported in 1921, indicated that many of the commonly used mechanical milkers were not then equipped with check valves that closed perfectly enough to prevent entirely the return of the condensation material; while this contamination did not seem to be large in amount in any of the machines tested it was of sufficient importance to be of real significance in the production of high grade milk and to make it difficult to secure the premiums for low count milk. A visit to 50 dairies in the districts in New York supplying high grade milk showed that there was no one thing so generally neglected in the care of milking machines as the cleaning of the check valves, stanchion hose and vacuum lines.

The condensation water in the vacuum lines provides a material in which bacteria and other micro-organisms may develop rapidly, especially during the warmer months. In many cases the odor is indicative of extensive bacterial action. Dirty material returning from the vacuum line may interfere with the proper closing of the check valves.

Bacterial flora of milking machines. Hastings and Hoffmann[53] compared the types of bacteria in machine and hand drawn milk by means of lactose litmus gelatin plates and noted no marked difference. Robertson[54] studied the organisms in milking machines by taking samples from various parts of the machines, including pipe lines and stanchion hose, by rinsing with sterile water, plating this out and then isolating the colonies which were predominant on the plates. The results secured indicate that certain relationships exist between the bacterial flora and the care given the machines. Gram

[50] Conn. (Storrs) Agr. Expt. Sta. Bul. 47. Part 1. 1907.

[51] Proc. Cert. Milk Prod. Assn. Am. 7th An. Conv. P. 28. 1914.

[52] N. Y. (Geneva) Agr. Expt. Sta. Bul. 488. 1921.

[53] See ref. 44.

[54] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 105. 1924.

negative rods and *S. lactis* were found quite regularly but were more prominent as the condition of the machines became less satisfactory. The white, gram positive cocci were the most common type living in the machines when a brine hypochlorite solution was used for treatment. The alkali forming rods appeared to be associated with the holding of tubes in cold water or old sterilizing solutions of inadequate strength. Spore formers were rarely isolated and only a few Escherichia-Aerobacter cultures were secured. Molds, which were primarily *Oidium lactis,* and yeasts were found in accumulations of old milk in various parts of the machines.

Quality of milk secured with machines under practical conditions. The quality of milk secured on farms using milking machines appears to be extremely variable. Certified and other milk that must meet rigid bacteriological requirements is sometimes drawn with machines so that there is ample evidence that low bacterial counts can be obtained, if proper methods are used in caring for the equipment.

Information from various sources shows that some machine drawn milk is of a very poor quality. Ruehle, Breed and Smith[55] reported that in connection with an investigation of the milk delivered to Geneva, New York, it was noted that six farms using milking machines ranked 17, 21, 33, 40, 41, and 42 among 44 farms on the basis of the bacteriological condition of the milk supplied; in a later period six farms among 36 ranked 6, 28, 29, 34, 35, and 36, on the same basis. In the dairy sections producing high quality butter it has been noted, in certain instances, that the installation of a milking machine on a farm has resulted in a decrease in the general quality of the milk or cream supplied; this is especially serious in connection with the milk used for the preparation of the butter cultures.

(3) The Coat of the Animal

The contamination of milk from the coat of the producing animal involves mainly the dirt falling into the milk during the milking process. This dirt includes manure, bedding, soil, dandruff, etc.; it carries organisms, often in considerable numbers, and these readily become distributed through the milk. The dirt ordinarily present in milk very largely gains entrance during the milking process and, for the most part, comes from the coat of the animal, although the air, the milker, and the utensils may also be sources.

The amount of dirt in milk. The amount of dirt in milk may be determined, either on a comparative basis by the use of cotton discs, or on an actual weight basis by collecting it through sedimentation or centrifuging or both; none of the methods recover all of the dirt present. Harding and Prucha[56] found that when the cows were unusually dirty and were milked into an open pail, the dirt in the unstrained milk amounted to 10.8 milligrams per quart, while with methods comparable to those used in the better class of dairies the amount of dirt was about 2.5 milligrams per quart. Under the former condition the increase in the bacterial content of the milk due to the dirt was about 17,000 per cc. These investigators found the bacterial content of the dirt from the coat of a clean cow to be 17,814,000 per gram while that from the coats of four dirty cows varied from 184,300,000 to 4,592,000,000 per gram; considerable variation in the bacterial content is to be expected because of differences in the nature of the dirt.

---

[55] See ref. 49.

Stocking[57] showed the influence of the dirt from the coat of the animal on the bacterial content of milk by determining the decrease in the count as a result of wiping the udder and flank with a damp cloth; 13 comparisons were made by alternating two groups of five cows each and milking into a covered pail. The results obtained are as follows:

Contamination of Milk by the Dirt from the Udder and Flank

	Bacteria per CC.		
	Total	Acid Producing	Liquefying
Udder and flank not wiped	7,058	3,554	81
Udder and flank wiped	716	185	47
Decrease due to wiping	6,342	3,369	34

Bacteria in cow manure. Prucha, Weeter and Chambers[58] collected 27 samples of fresh cow feces, held them at a temperature of about 23.9° C. (75° F.) and a relative humidity between 20 and 40 and determined the numbers of bacteria by the plate method at intervals during three weeks. The bacterial content of the fresh feces ranged from 434,000 to 73,200,000 per gram of dry matter and aver-

[56] Ill. Agr. Expt. Sta. Bul. 236. 1921.

[57] See ref. 11.

[58] Absts. Bact. 2. P. 6. 1918.

aged 8,486,000; the moisture content varied from 78.4 to 87.6 per cent and averaged 83.6. In all the samples there was a multiplication of the bacteria present. The rate of increase was very rapid during the first two days, after which it became slower but continued for several days until a maximum was reached. A slow decrease then began. The maximum number in nearly every sample reached several billion per gram of dry matter; in six it was between ten and eleven billion and in only one was it less than one billion. Many of the organisms present were gas formers.

Allen,[59] using the plate method, found that 50 samples of fresh cow feces contained from 160,000 to 634,000,000 organisms per gram, while 49 samples which had been dried on porous plates at 37° C. (98.6° F.) for 48 hours contained from 500,000,000 to 16,800,000,000 per gram. Representative colonies from the plates poured with the fresh feces were inoculated into litmus milk and the changes produced showed that a variety of organisms was present.

Solubility of cow manure in milk. Taylor[60] investigated the solubility of cow manure in milk and summarized the results as follows:

AVERAGE SOLUBILITY OF COW MANURE IN MILK

	Per Cent Moisture in Manure	Per Cent Solids in Manure	
		Soluble in Milk	Insoluble in Milk
Fresh manure	82.76	0.96	16.25
Air dried manure	5.74	5.22	89.04

This investigator found that manure is less soluble in milk than in water. The results also indicated that 91 per cent of the manure present in bottled milk will be visible at the bottom of the bottle.

Types of organisms from the coats of the cows. The types of organisms getting into milk from the coats of the cows are, in general, the types found in manure, soil, feeds and bedding. Many of them are very objectionable because of the rapid changes which they bring about in milk; the Escherichia-Aerobacter types are especially undesirable.

[59] Jr. Dairy Sc. 6. P. 479. 1923.

[60] Jr. Dairy Sc. 1. P. 303. 1917.

(4) The Milker

The milker may add organisms to the milk from the hands and clothing and also by coughing, sneezing and speaking over the utensils or the milk.

During milking, organisms are dislodged from the hands and often fall into the milk. The method of milking with the hands wet is very insanitary because some of the liquid, carrying dirt and bacteria from the hands as well as from the outside of the udder and teats, drips into the milk. Dirt may fall into the milk from dirty clothing, especially when a pail is carried so that the top brushes against it. The organisms coming from the mouth, throat, nasal passages, etc., of the milker are very objectionable because they may include pathogenic types.

(5) Miscellaneous Sources

The miscellaneous sources from which milk is contaminated include those that may be involved with certain lots of milk but not with others; examples are flies, cooling water splashing into the milk, visitors coming in contact with the utensils or milk, the dropping of a utensil which is then used without proper washing, etc. In some instances these miscellaneous sources of contamination may be of considerable significance; flies illustrate the possibilities along this line.

Flies may be responsible for the contamination of milk, either indirectly through contaminating utensils or directly by getting into the milk. In one series of experiments reported by Esten and Mason[61] in which 414 flies were examined, the number of bacteria present on the body of a fly varied from 550 to 6,600,000 and averaged 1,222,570. Armstrong[62] has also reported counts of the organisms that could be washed from the bodies of flies; seven flies caught in clean houses carried from 600 to 75,000 bacteria per fly and averaged 13,986, while six caught in dirty houses carried from 1,050 to 4,000,000 and averged 1,106.017. Because flies so commonly alight on fecal material, the organisms on their bodies include various objectionable types, especially the Escherichia-Aerobacter forms, which produce changes in milk and cream rapidly. In case human fecal material is exposed, there is the possibility of the typhoid organism and other intestinal pathogens being carried to the milk.

[61] Conn. (Storrs) Agr. Expt. Sta. Bul. 51. 1908.

[62] Am. Jr. Pub. Health. 4. P. 185. 1914.

Importance of the contamination from external sources on farms. The contamination which milk receives from external sources is ordinarily the important contamination to which it is subjected on the farms. The interior of the udder usually adds only small numbers of organisms and these are of types that produce changes in milk slowly, while the external sources may add large numbers, many of which cause changes in milk rapidly. As will be shown in the following chapter, the improvement that can easily be made in the quality of milk involves primarily a control of the external sources of contamination.

The extent of the contamination from external sources on the various farms depends on the conditions prevailing. Ayers, Cook and Clemmer[63] studied 249 samples of fresh milk from 12 farms before it was strained or cooled. The night milk averaged 115,135 per cc. and the morning milk 180,696, while the average for all was 135,146. A comparison of these counts with those obtained on milk drawn aseptically indicates that on the average farms the external contamination may involve large numbers of organisms.

CONTAMINATION SUBSEQUENT TO PRODUCTION

The contamination of milk subsequent to production may be just as extensive as that occurring on the producing farms. The use of poorly cared for equipment in dairy plants is especially serious and may add large numbers of organisms to milk. Vats, coolers, pumps, bottle fillers, etc., all present the same general problems as the cans and pails on the farms and the contamination from them may be extensive because the construction complicates the cleaning. The studies that have been carried out by the milk inspection departments in various cities have shown how serious the contamination of milk in plants may be. In some instances milk has been properly pasteurized and then so heavily contaminated during the cooling, bottling and handling, that the number of organisms was larger than in the original raw milk.

Coolers. Coolers often add considerable numbers of organisms to milk. Judkins and Downs[64] found the average bacterial content of six lots of milk after pasteurization in a vat was 439.5 per cc. with a minimum of 45 and a maximum of 965, while after cooling over a cooler the average count was 4,104 per cc., the minimum 1,000 and the maximum 9,580. Smith[65] reported the results of counts made at a milk plant over a period of five days; the average count on the pasteurized milk was 6,240 per cc. while that on the milk off the cooler was 8,452.

---

[63] U. S. D. A. Bul. 642. 1918.

[64] Conn. (Storrs) Agr. Expt. Sta. Bul. 99. 1918.

[65] See ref. 42.

Bottle fillers. Bottle fillers are one of the most important sources of bacteria in milk plants. Prucha, Weeter and Chambers[66] found that, when a bottle filler which had stood for 20 hours after washing was not steamed before use, there was a conspicuous increase in the bacterial content of the bottled milk; in ten trials in which the maximum count for the milk from the pasteurizer was 203 and the average 84 per cc., the maximum for the first bottle through the filler in each run was 209,600 and the average 96,900 per cc. The use of the filler gradually washed the organisms from the machine so that the contamination became less as the run progressed.

Judkins and Downs,[67] with equipment which was rinsed with scalding water before the processing operation began, noted that in six trials the pasteurized milk in the vat averaged 439.5 per cc., while the same milk at the bottler averaged 12,195 and in the bottles it averaged 48,033. The pump and piping taking the milk to the bottler accordingly added 11,755.5 per cc., while the bottling process added 35,838 more. Smith[68] carried out eight tests at a large milk plant and found the average count on the cooled milk was 2,038 per cc. while on the bottled milk it was 5,744; since the bottles at this plant were practically sterile the organisms must have largely come from the bottle filler. At a plant studied for 14 days the average count of the milk off the cooler was 21,750 per cc., that of the milk in the bottler was 28,305 while that of the bottled milk was 37,800. In another investigation Smith[69] found the average bacterial content of the milk off the cooler was 8,452 per cc. while the average count of the bottled milk was 15,592.

Homogenizers. Because of their complicated construction homogenizers are difficult to properly clean and care for and thus become an important source of contamination. The pressure under which the machines operate is probably a factor in forcing material into crevices from which it is difficult to remove by ordinary methods of washing; growth in this material between runs may cause unusually heavy contamination. Homogenizers have been studied most in connection with the contamination of ice cream mixes but are also of importance with the cream put out in many milk plants. Hammer and Sanders[70] noted that in the homogenization of an ice cream mix the homogenizer was sometimes the cause of contamination, even when it was given what was considered to be satisfactory care.

---

[66] See ref. 39.

[67] See ref. 64.

[68] See ref. 41.

[69] See ref. 42.

The study of the contamination from a homogenizer is complicated by the breaking up of clumps of organisms in the material run through the machine so that an increase in the numbers, as determined by the plate method, may not be due entirely to the addition of organisms. Running sterile water through the machine operated without pressure and plating this gives a more definite idea of the contamination than the regular homogenization of cream or an ice cream mix. In some instances the contamination is very clearly shown by the presence of species of organisms in the homogenized material that are entirely lacking in the unhomogenized material.

James[71] found that the influence of the breaking up of clumps of organisms during homogenization, on the bacterial content of cream, was fairly constant during the handling of one batch but that there was considerable variation between batches. Little difference was noted between pressures of 750 and 2,500 pounds per square inch which suggests that the lower pressure was sufficient to break up the clumps present. This investigator made five runs with pasteurized skim milk, using a machine that had been practically freed from organisms, and noted that the counts remained fairly constant during a run and that rehomogenization had little effect on the count. The comparative numbers of organisms before and after homogenization varied from a ratio of 1 to 1.5 to a ratio of 1 to 4.6.

If the homogenizer is a significant source of organisms, the contamination becomes less during a run, due to the gradual washing out of the organisms. In some instances the decrease is very rapid so that after the first few minutes of operation only small numbers of organisms are added from the machine.

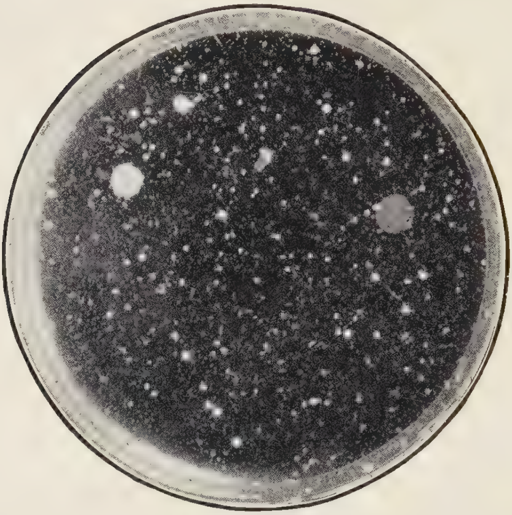
Influence of the season on the contamination from equipment. If equipment is so cared for that it is a source of organisms, there may be a seasonal influence on the extent of this contamination, due to the effect of temperature on the growth of organisms between the periods of use of the equipment. During the cold weather the multiplication of organisms may be negligible, while on the hot summer days it is certain to be extensive, if moisture is available.

Bottle caps. The bottle caps constitute a possible source of the

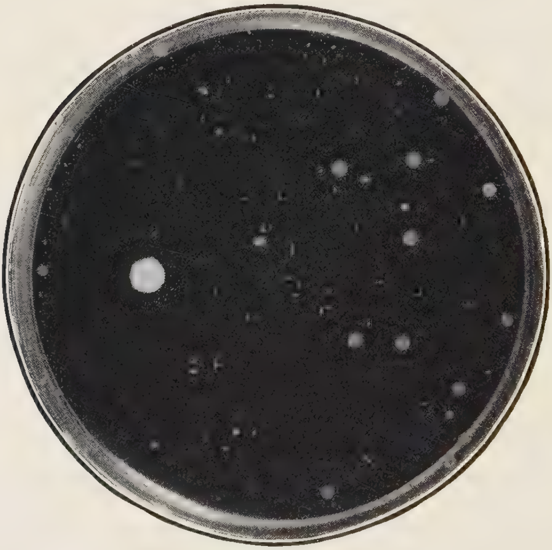
---

[70] Ia. Agr. Expt. Sta. Bul. 186. 1919.

[71] Thesis Iowa State College. Bacterial Contamination from the Homogenizer —its Control. 1923.



Colonies from 1/100 cc. of first material through the homogenizer.



Colonies from 1/100 cc. of material through the homogenizer after several minutes operation.

FIG. 21.—Contamination from Homogenizer.

contamination of milk and, when they are exposed to flies, dust and dirt, may be of considerable importance. Growth of organisms is not to be expected on bottle caps, provided they are stored in a dry place and, accordingly, the organisms present have been added directly.

Smith[72] found in the examination of 2,166 bottle caps, taken in lots of from 28 to 68 from the supplies in use at different pasteurization plants, that the organisms added per cubic centimeter of milk would have ranged from 0 to 453. The method of examination used was to wash a number of caps in sterile water, determine the bacteria in the water and then calculate to one-half the surface of a cap.

Data on the examination of bottle caps from Boston milk plants have been reported by the Health Department of that city at various times. In 1922, a total of 574 caps was examined[73] and divided into 287 lots, according to the dealers from whom they were obtained. Some of the caps were protected from dust and dirt and were designated "covered," while others were in open boxes or barrels were classified as "uncovered." The results secured are as follows:

Bacterial Content of Bottle Caps

Number of Lots of Caps	Protection	Lots under 50 Per Cap		Lots from 50 to 100 Per Cap		Lots over 100 Per Cap	
		Number	Average Count	Number	Average Count	Number	Average Count
86	Covered......	36	22	9	72	41	735
201	Uncovered....	84	25	22	78	95	956

These data suggest that the average bacterial content of exposed caps may be greater than of those kept covered.

Contamination from plant employees. The employees in plants handling milk and cream are a source of organisms comparable to the employees on dairy farms. Because the contamination of milk from human sources may include pathogens, it is especially serious when it occurs subsequent to pasteurization since this process is so regularly assumed to provide the protection desired. The modern large scale milk equipment, which almost entirely eliminates direct

[72] See ref. 41.

[73] City of Boston. An. Rpt. Health Dept. 1922. P. 63.

human contact with the milk after pasteurization, constitutes an important advance from the sanitary standpoint.

Contamination in the homes. After the delivery of milk and cream to the homes in which it is to be consumed there is still an opportunity for contamination. This comes especially from dishes and utensils and, in general, milk and cream should be left in the bottles employed for delivery until immediately before use. The practice of pouring bottled milk into a shallow pan and allowing it to stand so that it can be skimmed more easily is especially objectionable, not only from the standpoint of contamination but also because it permits the rapid absorption of the odors of other food products placed near the milk. The mixing of milk or cream from different lots and then holding often leads to souring and other changes, when these would not have occurred had one lot been used completely before the next was opened.

In some instances one consumer out of a considerable number complains that milk sours readily or undergoes some other objectionable change. This is commonly due either to serious contamination in the home or to a holding temperature that is very favorable for the growth of organisms.

With the types of bottle caps in common use there is an opportunity for the contamination of the lip of a bottle, especially from flies and from persons, and many of the organisms are carried into the milk when it is poured out. Jackley[74] has noted the presence of Escherichia-Aerobacter organisms on the tops of bottles of milk purchased on the market.

[74] Jr. Dairy Sc. 5. P. 406. 1922.

CHAPTER IV

REDUCING THE CONTAMINATION OF MILK

The methods of reducing the contamination of milk are suggested by the data available on the sources of the organisms and their relative importance. These methods may be divided into (1) those concerned with the production and handling of milk on the farms and (2) those involved in the handling of milk in dairy plants.

METHODS OF REDUCING THE CONTAMINATION OF MILK ON THE PRODUCING FARMS

Elimination of the fore milk. Since the fore milk contains more bacteria per cubic centimeter than the remainder of the milking, the elimination of the fore milk has been suggested as a means of reducing the contamination from the interiors of the udders and is practiced in some dairies interested in keeping the bacterial content of milk as low as possible. The usual procedure is to have someone go ahead of the milkers and draw the first few streams from each teat into a container, this milk then being discarded or used for feeding purposes. The fore milk should not be drawn on the floor since the fermentation there is likely to be responsible for undesirable odors in the stable.

The effect of eliminating the fore milk on the bacterial content of milk has been studied in eight comparisons by Stocking,[1] using two lots of five cows each and milking one group after rejecting the first three streams from each teat while, with the other, the fore milk was included; the two groups were alternated because of probable variations in the contamination from the interiors of the udders. Each procedure gave the lower count in four of the comparisons; the differences were sometimes considerable on a percentage basis, although the counts were all very low. The average counts are as follows:

---

[1] Conn. (Storrs) Agr. Expt. Sta. 18th An. Rpt. P. 66. 1906.

EFFECT OF REJECTING THE FORE MILK ON THE BACTERIAL CONTENT OF MILK

	Bacteria per CC.
Fore milk not rejected	522
Fore milk rejected	499
Difference due to rejecting the fore milk	23

The decrease in the bacterial count as a result of eliminating the fore milk amounted to approximately 4 per cent.

The influence of rejecting the fore milk was studied at the Iowa Agricultural Experiment Station by Glen Ellis with a somewhat different method. After carefully cleaning the udders and flanks of the cows, the fore milk and the remainder of the milking were each collected in a sterile container and the volumes and bacterial contents determined. From these data calculations were made as to what the bacterial content of the milk would have been had the two portions been mixed. The result showed that, on the average, discarding the fore milk reduced the bacterial count about 4 per cent.

Lamson [2] has reported data showing the effect of eliminating the fore milk that may be summarized as follows:

EFFECT OF REJECTING THE FORE MILK ON THE BACTERIAL CONTENT OF MILK

Cow Number	Milk Collected in Open Top Pail		First Two or Three Streams Discarded; Remainder Collected in Open Top Pail	
	Number Samples	Average Bacterial Count (Round Numbers)	Number Samples	Average Bacterial Count (Round Numbers)
136	23	5,600	14	3,100
155	8	4,700	40	6,800
159	8	3,200	9	2,600
129	8	8,600	11	7,800
163	16	7,900	9	6,700
128	21	3,800	27	3,300
195	12	6,400	6	3,000

---

[2] Md. Agr. Expt. Sta. Bul. 181. 1914.

With six of the seven cows the average counts were lower when the fore milk was excluded than when it was not and, in some instances, the differences were considerable on a percentage basis. The relationship between the average counts secured with cow 155 may have been influenced by the very great difference in the numbers of samples in the two groups.

From the results presented it appears that the elimination of the fore milk does not have a very significant influence on the bacterial content of milk, although it may be of importance when the counts are to be kept as low as possible. Another point to be considered is the flavor of the fore milk; this is frequently rather unsatisfactory and discarding the fore milk may be an advantage, if milk with an exceptionally fine flavor is desired. The rejection of the fore milk has its influence on the volume of milk to be marketed but involves little fat loss because this portion of the milking is comparatively low in fat.

Elimination of cows giving high count milk. In the production of milk that must comply with rigid bacterial standards, cows that regularly give milk with high counts are undesirable and should be removed from the producing herd. They can be detected only by making counts on samples from the individual animals.

Effect of various treatments on the bacteria in the udder. Atwood and Giddings[3] attempted to reduce the contamination of milk very carefully drawn. After the udder had been thoroughly stripped, the end of the teat was dried with a sterile towel and then moistened with 25 per cent lysol. This was wiped off, carbolated vaseline rubbed on and the vaseline protected with adhesive tape. The counts obtained on the milk after such treatment were not significantly different than those obtained on milk produced normally, which indicates that contamination through the teat canal has little influence on the number of organisms coming from the interior of the udder. The injection into the udder of materials intended to destroy or inhibit the organisms caused inflammations.

Hoffmann[4] injected udders with physiological salt solution, 2 per cent boric acid solution or distilled water and found that such inert materials produced a rapid and marked leucocytosis. In some instances an increased leucocyte count was accompanied by a decreased bacterial count, which may have been due to phagocytic action since microscopic preparations showed many leucocytes containing from 5 to 25 bacteria, while leucocytes from uninjected quarters showed no such activity.

---

[3] W. Va. Agr. Expt. Sta. Bul. 134. 1911.

[4] Wis. Agr. Expt. Sta. Res. Bul. 6. 1909.

Limiting the dust in the stable air. Although the stable air is not a source from which large numbers of organisms get into milk, an attempt to limit the contamination from it is advisable. This involves primarily a careful selection of the time of such operations as brushing, feeding and bedding the animals so that the air will be reasonably free from dust during the milking. With an excessive amount of dust in the air considerable material settles into the milk and has an influence on the amount of visible dirt as well as some effect on the number of micro-organisms.

Proper care of utensils. Since utensils may be responsible for the addition of large numbers of bacteria to milk, their proper care is necessary in the production of milk with a low count. Such care consists of thorough washing, the destruction of practically all of the organisms present and, finally, the drying so that organisms will not have an opportunity for growth in the water and traces of nutrients present in the undried utensils.

Washing of the utensils should begin with the rinsing out of the milk with cold or only slightly warmed water; hot water often causes some of the milk constituents to stick to the utensils and these materials are then removed with considerable difficulty. After rinsing, the utensils should be scrubbed with hot water containing washing powder. The use of soap is not advisable because it is more difficult to remove than washing powder and traces left may give the milk a soapy taste. The wash water must be reasonably clean or it may be a source of organisms. After careful washing, thorough rinsing is again necessary and this should be followed by steaming or scalding with hot water. Unless some special method of drying is to be used, the metal should be heated so thoroughly by the steaming or scalding that the water remaining after the utensils have been carefully drained will evaporate. Escape of the water vapor is greatly facilitated by leaving the utensils upright; when they are inverted on racks, the vapor rises and, since it can not readily escape, it is condensed and much of the water remains. Utensils should not be dried with a cloth because of the possibility of adding large numbers of organisms. The inversion of the dry utensils in a clean atmosphere is an aid in limiting air contamination. Harding, Ruehle, Wilson and Smith[5] found that protection of thoroughly steamed milk pails against accidental contamination had a measurable effect in reducing the bacterial content of the milk.

---

[5] N. Y. (Geneva) Agr. Expt. Sta. Bul. 365. 1913.

The mechanical equipment which washes, steams and dries cans is very satisfactory when the number of cans handled justifies the expense. The drying of the cans is important, entirely aside from the bacteriological considerations, for two reasons: first, cans left with moisture in them rust and deteriorate more quickly than dry cans and second, cans containing moisture in which organisms may multiply often develop undesirable odors and these are quickly taken up by milk or cream. Ayers and Mudge[6] have considered the possibility of sterilizing utensils with hot air. They found that raising the temperature to 120° C. (248° F.) during a four minute period destroyed a great many bacteria but did not give uniformly low results, while an added holding period of two minutes at 120° C. increased the destruction. When a temperature of 140° C. (284° F.) was reached in four minutes, a high destruction of organisms was secured and holding at this temperature increased it.

Utensils may be sterilized by putting them in a cabinet or container[7 and 8] in which they can be subjected to heat. Where steam is available, a cabinet into which the steam can be turned is very useful, while a metal container in which steam can be generated over a fire is also satisfactory; equipment of the latter type may be used to heat water for washing purposes as well as for the sterilization.

Prucha and Harding[9] have shown that rinsing cans removes some of the organisms mechanically and that, as the temperature is increased, the destruction of organisms begins. Small amounts of water are quickly cooled when added to a can and quantities carrying sufficient heat to properly raise the temperature of the metal are necessary. Harding, Prucha, Weeter and Chambers[10] have presented data indicating the necessity of using sufficient steam if the proper destruction of organisms is to be secured during the steaming of cans.

The influence of sterilizing the utensils in reducing the bacterial content of milk is illustrated by the findings of Ayers, Cook and Clemmer[11] in a comparison of sterile pails and cans with those in an unsterile condition. Throughout the trials the floors and cows were dirty, the manure being removed only weekly. The milk representing the unsterile utensils was secured from July 22 to August 14 inclusive, while that representing the sterile utensils was obtained from September 14 to October 7 inclusive. The summarized results are as follows:

---

[6] Jr. Dairy Sc. 4. P. 79. 1921.

[7] U. S. D. A. Farmers' Bul. 1473. 1926.

[8] Prov. B. C. Dept. Agr. Dairy Cir. 13. 1926.

[9] Ill. Agr. Expt. Sta. Bul. 230. 1920.

[10] Jr. Dairy Sc. 5. P. 282. 1922.

[11] U. S. D. A. Bul. 642. 1918.

INFLUENCE OF STERILE UTENSILS ON THE BACTERIAL CONTENT OF MILK

	Utensils Not Sterile		Utensils Sterile	
	Number Samples	Average Number Bacteria per CC.	Number Samples	Average Number Bacteria per CC.
Open pail..........	26	497,653	36	22,677
Small top pail.......	28	368,214	36	17,027

The data given show that a greatly reduced bacterial count accompanied the sterilization of the utensils, even under the otherwise unsatisfactory conditions of production. While the counts were lower with the small top pail than with the open pail, the sterilization of the utensils was of much more importance than the type of pail.

Ayers, Cook and Clemmer[12] made a more direct determination of the effect of sterile utensils on the bacterial count by securing data on milk from a sterilized, small top pail and from one that was unsterilized, the milk from two cows being secured with each pail. The cows were cleaned and bedded but the udders were not washed. Sixty samples from the sterile pail showed an average bacterial content of 6,306 per cc. with a maximum of 21,500, while 59 samples from the unsterilized pail averaged 73,308 with a maximum of 284,000. In other trials carried out the cows were allowed to get dirty and the manure was removed only twice a week. Thirty samples from sterile utensils averaged 31,040 bacteria per cc., while 50 samples from utensils that were washed directly after milking, but not sterilized, averaged 666,520 per cc.; in 20 trials the utensils were held eight hours before washing and the counts then averaged 1,667,000.

The influence of unsterile utensils on the bacterial content of milk is extremely variable and depends on such factors as the care used in the washing, the heating incident to the washing and the opportunities for the growth of organisms between washing and use. On farms where no attempt is made to give the utensils proper care, large numbers of organisms commonly come from them, especially during the warm months when there is rapid growth in the moisture present.

---

[12] See ref. 11.

Construction of utensils. The proper care of utensils is only possible when they are in a satisfactory condition. With tin utensils all seams should be well filled with solder and there should be no rough areas, such as may be formed when the tin wears away and exposes the iron beneath. A perfectly smooth surface, so that there is no tendency to hold traces of milk solids or collect organisms, is the most desirable.

Care of milk coolers, metal strainers and separators. The coolers, metal strainers and separators used on producing farms may be classed with the utensils since they provide the same general opportunities for the contamination of milk. Thorough washing, heating and drying are necessary with them and should be followed by proper protection from dust. Steaming or scalding just before use may be practiced, if the previous handling has not been entirely satisfactory.

Care of milking machines. The care of milking machines involves two distinct procedures, the washing and the sterilization; the sterilization refers to the practical sterility advisable with dairy equipment rather than absolute sterility.

The washing should be carried out as soon as the milking is completed by drawing through each unit a pail of cold water, then a pail of hot water containing washing powder and, finally, a rinse of hot water. The teat cups should be doused in and out of the water so that water and air are alternately drawn through the unit and the removal of the milk aided in this way. The outside of the teat cups and rubber tubes should then be cleaned. About twice a week the teat cups and tubes should be taken apart and carefully scrubbed with hot water and washing powder.

The sterilization of the teat cups and tubes is usually carried out either by the use of a chemical or by the application of heat. Sometimes a combination of the two methods is employed, the parts being held in a solution of some chemical after being heat treated.

With the chemical method, the teat cups and tubes are put into a sterilizing solution and left until the next milking. The solution may be made from various reagents but hypochlorites have been most widely employed.

A hypochlorite solution can be prepared by adding a gallon of water to the contents of a 12 ounce can of chloride of lime in an earthenware jar; a paste is first made and then the remainder of the water added with thorough stirring. The clear solution that collects on standing contains a high concentration of hypochlorite and is the material desired. It may be kept in a covered jar or transferred to a bottle and held stoppered. Chloride of lime deteriorates with extended holding so that the preparation from which the hypochlorite solution is made should be fresh.

A sterilizing solution in which the teat cups and tubes may be kept is made by placing 50 pounds of salt in a 20 gallon jar, filling with water to within several inches of the top and then, when the salt is dissolved, adding one quart of the hypochlorite solution. Additional hypochlorite solution must be added from time to time, a pint at least once a week in winter and twice a week in summer, to maintain the strength. The salt keeps down the growth of many bacteria and thus prolongs the usefulness of the solution. Once each month, or oftener if the preparation tends to become dirty, a new solution should be made up. When the tubes are put into the solution air must not be trapped in them since this leaves portions of the tubes that are not subjected to the sterilizing action. The jar containing the solution should be kept well covered. Commercial hypochlorites may be used in place of the solution made from chloride of lime and certain other chemicals are also satisfactory. In some instances a fresh solution is made up for each treatment of the teat cups and tubes and the salt then omitted. The rinsing out of the teat cups and tubes, shortly before use, by drawing hot water through them largely removes the sterilizing agent present.

With the chemical method of sterilization it is very necessary that the solutions employed be kept up to an active strength. In many instances high count milk has been due to the employment of preparations in which the sterilizing agent has fallen to such a low concentration that the desired destruction of organisms is not secured. This may be the result of the using up of the reagent by the organic matter carried into the solution but very often the original concentrations are too low.

The heat method of treatment consists of immersing the teat cups and tubes in water and raising the temperature with steam or a fire. The value of this procedure was pointed out by Hart and Stabler[13] in California, in 1920; they concluded that heat sterilization is the only way to successfully treat milking machine rubber parts under ordinary ranch conditions. The heat has an effect on the life of the rubber parts but, if care is used to prevent excessive temperatures, this need not be serious. A temperature of 71.1° to 76.7° C. (160° to

---

[13] Jr. Dairy Sc. 3. P. 33. 1920.

170° F.) for about 30 minutes appears to give a satisfactory destruction of organisms. The parts may be left in the water until the next milking or various other procedures may be followed. Burgwald[14] found that heating a unit in water at from 71.1° to 75° C. (160° to 167° F.) for from 20 to 35 minutes and then removing it to a refrigerator and keeping it there between milkings increased the length of life of the rubber parts over those left in the hot water and resulted in counts which were only slightly higher than those of milk drawn with the equipment left in the water. Holding the heat treated unit in a weak chlorine solution between milkings gave counts that were still lower than those obtained with the unit held in the water. The effect on the bacterial content of the milk, of the method of holding the unit after the heat treatment, is shown in the following summary which includes winter and summer results:

BACTERIAL COUNTS OBTAINED WITH HEAT TREATED MILKING MACHINE UNITS HELD IN VARIOUS WAYS

Method of Holding Following Heating	Number of Samples	Bacteria per CC.	
		Range of Counts	Average Number
Placed in refrigerator	296	300 to 10,900	3,110
Placed in weak chlorine solution	292	200 to 9,300	2,200
Remaining in hot water	290	300 to 7,400	2,570

Burgwald also compared temperatures of 62.8° to 65.6° C. (145° to 150° F.) and 71.1° to 73.9° C. (160° to 165° F.), the units being left in the water between milkings. With the lower temperatures the life of the rubber parts was greater than with the higher temperatures but the bacterial counts were not as low; 142 samples showed a range of counts from 3,000 to 51,000 per cc., with an average of 11,930 while 40 counts secured on milk drawn following the use of the higher temperatures ranged from 700 to 6,000 and averaged 2,520. A method of holding the unit in water at 71.1° to 73.9° C. for from 20 to 45 minutes and then hanging it in a warm room was also tried; 154 samples of milk secured in this way varied from 1,100 to 24,000 per cc. and averaged 5,540. With heat treatment there was a tendency for the rubber tubing to stick to the metal parts unless the units were taken apart rather frequently.

---

[14] Jr. Agr. Res. 34. P. 27. 1927.

Fisher and White[15] found that sterilization with steam was very destructive of the rubber parts, while with hot water treatment at 93.3° C. (200° F.) or above for one-half hour the damage to the rubber was not prohibitive and the counts on the milk were uniformly low.

The milk pails and lids require careful washing and steaming or scalding, if contamination from them is to be controlled. Devices to prevent the return of moisture from the vacuum line must be kept in a satisfactory condition and the stanchion hose should be properly cleaned to reduce the possibility of serious contamination from it.

The holding of the washed teat cups and tubes in a container into which cold water is allowed to run continuously has been tried as a means of keeping milking machines in a satisfactory condition bacteriologically. Ruehle, Breed and Smith[16] found that as long as the water was below 18° C. (64.4° F.) it effectively controlled bacterial growth but that as soon as it reached this temperature a change occurred and much larger counts appeared. Robertson, Finch and Breed[17] noted that tubes held in cold running water eventually became coated with large numbers of water bacteria, even when the temperature was lower than 14.4° C. (58° F.); accordingly, they did not regard it as an entirely satisfactory method of caring for tubes. Fisher and White[18] reported that a stream of water, at temperatures well below 12.8° C. (55° F.), running through the units between milkings was a convenient and reliable means of producing good quality milk but that with high water temperatures the method failed.

Cleaning of the cows. The control of the contamination from the bodies of the cows requires that the animals be kept reasonably clean. This involves frequent brushing and, in the case of farms producing a special grade of milk, washing or wiping the udders and flanks; occasionally, more complete washing of the animals is practiced. The method of bedding and the type of stanchion are important factors in preventing the coats of the animals from accumulating manure.

Stocking[19] studied the effect of wiping the udders and flanks with a damp cloth, on the bacterial content of milk, by using two groups

[15] Conn. (Storrs) Agr. Expt. Sta. Bul. 144. 1927.

[16] N. Y. (Geneva) Agr. Expt. Sta. Bul. 450. 1918.

[17] N. Y. (Geneva) Agr. Expt. Sta. Bul. 492. 1922.

[18] See ref. 15.

[19] See ref. 1.

of five cows each and milking one group with such wiping and the other without; the groups were alternated to eliminate variations in the contamination from the interiors of the udders. A small top pail was used and the milk from the five cows in a group was mixed before sampling. In all but one of the 13 comparisons the milk from the wiped animals had the lower count; often the difference was small but, in some instances, it was rather large. The average count following wiping was 716 per cc. while without wiping it was 7,058.

Ayers, Cook and Clemmer[20] secured data showing the influence of cleaning the cows and floors on the bacterial content of milk; the main effect of clean floors undoubtedly was in keeping the animals clean. The samples from the dirty animals were secured from April 11 to May 6 inclusive and those from the clean animals from June 5 to June 13 inclusive. The summarized results are as follows:

Effect of the Condition of the Cows on the Bacterial Content of Milk

	Cows and Floor Dirty, Manure Removed Weekly, Utensils Sterile		Cows and Floor Clean, Manure Removed Daily, Udders and Teats Washed, Utensils Sterile	
	Number Samples	Average Number Bacteria per CC.	Number Samples	Average Number Bacteria per CC.
Open pail	41	86,212	15	4,947
Small top pail	41	24,439	15	2,667

The reduction due to the cleaning of the animals and the washing of the udders and teats is very pronounced. In additional investigations Ayers, Cook and Clemmer studied the effect of washing the udders and teats on the bacterial content of milk by having four cows cleaned and bedded, the floor clean and the manure removed daily and then milking two of the cows after the udders and teats had been washed while the other two were milked without such washing; the utensils employed were sterile. The 65 samples from the washed udders averaged 2,154 per cc. and had a maximum of 5,400, while the 65 from the unwashed udders averaged 4,524 per cc. and had a maximum of 20,400. From these data it is evident that, even when the animals are in such a condition that contamination from the bodies is slight, the washing of the udders and teats brings about considerable reduction in the bacterial content of the milk.

---

[20] See ref. 11.

Use of small top pails. Small top or sanitary milk pails are intended to reduce the area through which dust and dirt can fall into the milk and, in this way, to decrease contamination from the body of the animal and from the air. They are very helpful in the production of clean milk and are widely employed. A number of types have been devised but a simple construction is advisable so that the pails can be washed and used without inconvenience.

Stocking [21] noted large reductions in the bacterial content of milk as a result of using small top pails; under dirty stable conditions the counts secured with one type were only 3 per cent as high as those obtained with an open pail. Harding, Wilson and Smith [22] found that more than one half of the contamination of milk could be prevented by the use of small top pails; six types of pails studied by them gave reductions from 48.4 to 70.1 per cent.

North [23] has reported data showing the influence of small top pails, with and without extra care in sterilization, on the bacterial content of milk; these are as follows:

INFLUENCE OF SMALL TOP PAILS ON THE BACTERIAL CONTENT OF MILK

Farm	Bacteria per CC.		
	Open Pails. Feb. 15 to Mar. 14	Small Top Pails. Mar. 15 to Mar. 31	Small Top Pails, Extra Care in Sterilization. Apr. 1 to Apr. 15
1	258,000	89,000	31,600
2	541,000	185,000	50,000
3	278,000	35,000	17,600
4	445,000	113,000	11,300
5	237,000	32,000	17,500
6	821,000	380,000	99,000
Average	430,000	139,000	37,830

The counts show very significant decreases in the numbers of bacteria as a result of using small top pails. The same general reduction is evident in the following data, also presented by North, which are of special interest because they were secured on a daily production of about 3,000 quarts of milk, coming from 28 farms and delivered to one shipping station.

---

[21] Conn. (Storrs) Agr. Expt. Sta. 19th An. Rpt. P. 77. 1907.

[22] N. Y. (Geneva) Agr. Expt. Sta. Bul. 326. 1910.

[23] Am. Jr. Pub. Health. 7. P. 25. 1917.

INFLUENCE OF VARIOUS FACTORS ON BACTERIAL CONTENT OF MILK

	Bacteria per CC.	Remarks
Dec. 26	5,500,000	Bacterial tests begun.
31	3,000,000	
Jan. 6	1,000,000	
13	500,000	Freezing weather.
29	800,000	
Feb. 12	900,000	
Mar. 5	700,000	
12	250,000	Small top pails distributed.
19	250,000	
26	80,000	Pails and cans sterilized.
Apr. 2	100,000	
9	70,000	
16	35,000	Premium paid for low count.

Ayers, Cook and Clemmer[24] compared the results obtained with open and with small top pails under different conditions of production and the results are summarized in the following table; some of the values included have already been given in connection with the influence of other factors on the bacterial content of milk.

INFLUENCE OF SMALL TOP PAILS ON THE BACTERIAL CONTENT OF MILK UNDER VARIOUS CONDITIONS

Conditions of Production	Open Pails		Small Top Pails	
	Number of Samples	Average Bacteria Per CC.	Number of Samples	Average Bacteria per CC.
Cows and floor dirty, manure removed weekly, utensils not sterile	26	497,653	28	368,214
Cows and floor dirty, manure removed weekly, utensils sterile	36	22,677	36	17,027
Cows and floor dirty, manure removed twice a week, udders and teats washed, utensils sterile	18	6,166	23	2,886
Cows and floor dirty, manure removed weekly, utensils sterile	41	86,212	41	24,439
Cows and floor dirty, manure removed weekly, utensils not sterile	36	153,905	36	114,497
Cows and floor clean, manure removed daily, udders and teats washed, utensils sterile	15	4,947	15	2,667

---

[24] See ref. 11.

Under all the conditions studied, the milk drawn in small top pails had a lower bacterial count than that drawn in open pails and commonly the difference was pronounced.

The reduction in the bacterial content of milk, as a result of the use of small top pails, is extremely variable and depends especially on such factors as the condition of the body of the cow and the amount of dust in the air. Under unsatisfactory conditions of production the number of organisms kept out of the milk per cubic centimeter is larger than under good conditions but in both cases the reduction on a percentage basis is considerable.

Reducing the contamination from the milker. The reduction of the contamination from the milker involves mainly a decrease in the material that gets into the milk from the hands, clothing and mouth and nasal passages. Clean hands add fewer organisms than dirty ones and, in some dairies interested in the production of milk of an exceptionally high sanitary quality, the hands are washed before each cow is milked. The milk should not touch the hands since, if it does, it tends to wash off dirt and bacteria that may be present. Clean clothing is very desirable because the dirt falling from unclean garments may get into the milk. The principal advantage of wearing white suits around dairy products is that they quickly show an accumulation of dirt. Speaking, coughing or sneezing with the mouth over the milk may add considerable numbers of organisms. Such contamination is especially objectionable because of the possibility of pathogenic species from the nose, throat or lungs being included.

The extent of the contamination from the milker is largely determined by the general habits of the individual. Persons doing milking should be neat and clean and should appreciate the importance of careful methods in the handling of materials intended for human consumption. Such practices as working in a hot, dusty field and then milking without changing the clothing or washing the hands are very objectionable and must be eliminated, if the confidence of the consumers of milk is to be retained.

Influence of the stable on milk contamination. At one time the stable was thought to be an important factor in the production of milk low in bacteria and a poor stable has often been offered as an excuse for a high bacterial count in milk. The investigations that have been carried out, however, have shown that milk of a high sanitary quality can be produced in a stable of a very poor type.

North[25] studied, for a year, the sanitary quality of the milk

[25] See ref. 23.

produced in two barns of very different construction and surroundings, by running counts at least three times a week and often daily. In both barns very careful methods were used but, with the old barn, the cow yard, lighting, ventilation, floors, walls and ceiling were in a most unfavorable condition. The bacterial content of the milk from the sanitary barn averaged 1,097 per cc. with monthly averages from 287 to 3,300 while, with the milk from the old barn, the average was 3,102 per cc. and the monthly averages from 331 to 10,200. From these results it is evident that milk with a very low bacterial count can be produced in a stable that would ordinarily be considered as unsatisfactory.

An interesting observation was made in connection with the cream improvement work carried out among the patrons of an Iowa creamery; the cream was not examined for its bacterial content but was judged on the basis of the flavor and aroma. One patron, whose cream was of the very highest quality, was found to be using a stable consisting of a pile of straw thrown over a framework of poles and which would ordinarily be considered as a poor place for the production of high grade milk or cream.

Harding, Ruehle, Wilson and Smith[26] studied the influence of different conditions of the walls and ceiling of a barn on the bacterial content of the milk, using six milkings from each of six cows for each condition; the bacterial content of the milk before plastering the barn averaged 775 per cc., that after plastering averaged 889 and that after painting the walls averaged 656. The influence of whitewashing was also studied by using 20 samples from each of three cows, both before and after whitewashing; the milk secured before the stable treatment averaged 953 per cc. and that secured after the treatment averaged 1,193.

Influence of clipping the udder and flank. The influence of clipping the udders and flanks of the producing cows on the bacterial content of milk seems to be negligible. Harding, Ruehle, Wilson and Smith,[27] using two cows and a number of milkings from each, found that the bacterial content of the milk before clipping the udders and flanks was 231 per cc. while after clipping, it was 331. In another trial, involving 22 samples from each of four cows before clipping and 24 samples from each after clipping the udders, flanks and tails, the average count before clipping was 204 per cc., while after clipping it was 320.

Important factors in producing clean milk. The factors of im-

[26] See ref. 5.

[27] See ref. 5.

portance in producing milk with a low bacterial count are sterile utensils, reasonably clean cows and small top pails and these form the basis for any contemplated improvement in the sanitary quality of milk. None of the factors involve either an unreasonable effort or prohibitive expense, but do require an understanding of the care necessary in the production of a material secured under the conditions obtaining with milk. The incentive to produce milk of a good sanitary quality, that has been provided by certain companies in the form of a bonus for milk having a low bacterial count, has shown what can be accomplished under ordinary conditions.

Ayers, Cook and Clemmer[28] studied the effect of sterile utensils and small top pails, in reducing the bacterial content of milk or cream, on a number of farms during the summer when the cows were on pasture most of the time and their coats practically free from visible dirt; counts were first obtained for a considerable period on milk produced in the ordinary way and then, for a time, on that produced with sterile, small top pails. The results are given in the following summary:

INFLUENCE OF STERILE, SMALL TOP PAILS ON THE BACTERIAL CONTENT OF MILK WHEN THE COWS WERE REASONABLY CLEAN

Farm	Open Pails, Utensils Not Sterilized		Small Top Pails, Utensils Sterile	
	Number Samples	Average Number Bacteria per CC.	Number Samples	Average Number Bacteria per CC.
1	19	116,384	16	10,637
20	18	15,050	16	4,656
27	19	186,995	16	3,606
29	19	77,095	16	1,962
43	18	34,861	16	2,150
178 *	16	49,181	16	3,025

* Cream produced, separator parts sterilized.

The data show large reductions in the bacterial counts as a result of using sterile, small top pails instead of unsterilized, open pails.

North[29] has presented data illustrating the effect of sanitary methods in decreasing the bacterial content of milk produced on Pennsylvania farms. The milk drawn by the usual milkers was first studied and then milkers experienced in careful methods were brought to the farms. The imported milkers employed the old equipment, except that they used small top pails, and were on the farms not more than two hours. The bacterial counts on the milk secured by the two groups of milkers are as follows:

---

[28] See ref. 11.

[29] North, Charles E. Farmers' Clean Milk Book. P. 113. 1918.

INFLUENCE OF SANITARY METHODS ON THE BACTERIAL CONTENT OF MILK

Farm	Bacteria per CC.	
	Milk Drawn by Usual Milkers April 5	Milk Drawn by Imported Milkers April 6
A	1,830,000	3,300
B	1,520,000	3,100
C	4,830,000	4,600
D	4,000,000	7,000
E	1,450,000	4,100
F	3,600,000	61,000
G	60,000	800
H	. . . . . .	2,500
I	70,000	1,600
J	500,000	5,600

The decrease in the bacterial content of the milk resulting from the use of careful methods is very striking.

The influence of sanitary methods on the bacterial content of large volumes of milk is shown by the following data secured by North:[30]

INFLUENCE OF SANITARY METHODS ON THE BACTERIAL CONTENT OF MILK

City	Number Dairymen	Quarts Daily	Bacteria per CC.	
			Before Sanitary Methods Were Used	After Sanitary Methods Were Used
Homer, N. Y......	70	12,000	500,000	10,000
Sparks, Md.......	28	3,000	5,000,000	40,000
Oxford, Pa........	135	10,000	2,000,000	40,000
Kelton, Pa........	56	4,000	3,000,000	25,000
Fair Haven, Vt....	20	1,200	1,500,000	25,000

---

[30] See ref. 23.

These methods involved (1) milking with clean, dry hands into small top pails from udders free from loose dirt, (2) sterilization of pails, cans, strainers, etc., with boiling water, and (3) cooling milk by submerging cans in tanks of spring or ice water.

In another comparison North found that the fresh morning milk of a large shipping station patronized by over 90 farmers, averaged 393,000 bacteria per cc. for August and September, 1914, while, after the introduction of sanitary methods, the average for August and September, 1915, was 55,000.

The results that have been secured under widely varying conditions indicate that anyone who will use a reasonable amount of care can produce milk with a fairly low bacterial count. Elaborate equipment is not needed but an understanding of the methods of preventing contamination from the important sources is very necessary. Careful production, combined with prompt cooling to low temperatures, insures the delivery of milk which will have a low bacterial count and which, accordingly, will be free from abnormal flavors and aromas of bacterial origin and will keep satisfactorily.

METHODS OF REDUCING THE CONTAMINATION OF MILK AND CREAM IN DAIRY PLANTS

Since the equipment is the most important source from which micro-organisms get into milk and cream in dairy plants, careful attention should be given to everything with which these products come in contact. The principles involved in caring for equipment consist of (1) careful washing to remove milk solids as completely as possible, (2) steaming to destroy practically all the organisms present, (3) drying so that there will be no moisture left in which organisms may grow and (4) protection against the addition of organisms from various sources. In general, the contamination from the air in dairy plants is of little importance and equipment that has been properly cleaned and dried is not likely to be seriously contaminated on standing a short time. However, in store rooms, coolers, etc., where there may be a high humidity, the development of molds often results in the presence of considerable numbers of spores in the air.

Washing of equipment. The washing of dairy plant equipment involves rinsing with cold or warm water, scrubbing with hot water containing washing powder and then careful rinsing with hot water. The scrubbing is a step in the process that is often poorly done. In pasteurizing equipment, in the upper parts of coolers and wherever milk or cream is exposed to high temperatures, there is more than the usual tendency for the solids to stick to the metal and, without thorough scrubbing, the cleaning will be incomplete. If careless treatment continues there is a gradual accumulation of material that interferes with the heat transfer, develops objectionable flavors and aromas and may make possible the growth of organisms. The scrubbing should not be limited to exposed surfaces but should include every part of the equipment that is accessible to a brush.

Steaming of equipment. The carefully washed equipment should be steamed at once. The steaming must be thorough so that all the parts are well heated and so that, with complete drainage, the remaining moisture will be evaporated. In some plants the steaming is so poorly done that the expected results can not be accomplished. When the steam can be largely confined, as in a vat or an internal cooler, the metal is easily heated through but an exposed surface requires more attention, if the work is to be properly done. It must be recognized that it is not the presence of steam but the heat supplied by it that accomplishes the destruction of the organisms and that the raising of the temperature of the equipment to the desired point can not be accomplished, except when the proper time factor is provided.

Drying of equipment. Although there are exceptions, with many pieces of equipment the proper steaming supplies heat enough to evaporate the water that can not be drained away. The draining should be going on during the steaming and, as soon as the steaming is complete, the water vapor should be permitted to quickly escape. If this is not done it is probable that moisture will still be present when the temperature has dropped to a point where evaporation is negligible and there will then be an opportunity for the growth of organisms in the equipment before the next period of use.

Equipment that is difficult to care for. Because of the construction, some of the equipment in dairy plants is very difficult to keep in a sanitary condition and commonly adds large numbers of organisms to milk or cream with which it is used. The homogenizer is an extreme example of such equipment and has been repeatedly responsible for contamination so extensive that the keeping qualities of table cream have been seriously influenced. Bottle fillers also require careful treatment if they are to be kept in a satisfactory condition.

In the cleaning of equipment having a complicated construction the dissembling during washing should be as complete as possible and the parts should be thoroughly scrubbed. A hot solution of washing powder run through the equipment can not take the place of brushing, as is evident from the accumulations of milk solids and the odors so commonly noted when poorly cared for equipment is taken down. After the scrubbing, the parts should be rinsed, steamed and dried as completely as possible. Whenever the steam must pass through an extended system of any sort, the steaming should be continued until the entire metal surface has been thoroughly heated. In some instances in which the steaming of equipment has apparently yielded unsatisfactory results from the bacteriological standpoint, it has been found that more extended steaming gave the desired destruction of organisms. Whenever the effectiveness of the treatment given is open to question, the equipment should be steamed or rinsed with hot water just before use. Many of the organisms not readily destroyed by heat may be washed out in this way, especially when large volumes of water are used.

Importance of the condition of the cans returned to the farms. The cans returned to the farms from some of the dairy plants are in a condition that is entirely unsatisfactory for the transportation of milk or cream. In certain instances this is due to careless washing, while in others, the washing is well done but moisture is left so that the growth of organisms may occur. Moisture is especially serious in cans that are shipped for considerable distances and exposed to summer temperatures. Unless the cans returned to the farms are in a satisfactory condition it is difficult to convince the farmer that the methods of caring for utensils are important.

CHEMICAL STERILIZATION OF DAIRY EQUIPMENT

Steam and hot water are the agents commonly used in the destruction of organisms in dairy equipment and they have proved very effective. Chemical sterilization is also employed and it provides a means of treatment that may be useful under certain conditions, for example, when hot water and steam are inadvisable or unavailable.

Chemicals have been quite generally applied to the sterilization of milking machine parts. The early methods employed in maintaining milking machines in a satisfactory condition, bacteriologically, involved the use of solutions for the teat cups and rubber tubes and the methods of heating were developed later. The chemical treatment of milk bottles in small plants[31] has also proved useful in certain instances where equipment for treating in washing machines or steam chambers was not available.

---

[31] An Apparatus for Sterilizing Milk Bottles at Small Milk Plants and Dairies. Minn. State Bd. of Health. Div. of Sanitation. 1924.

The chemicals that appear to be best suited for use in connection with dairy equipment are the chlorine derivatives, including chlorine gas, hypochlorites and chloramines. The hypochlorites have been widely used and are valuable because they actively destroy microorganisms, have an odor that is not objectionable and are non-poisonous. In some instances they have been combined with washing powders to increase the destruction of organisms during the washing process.

Unless dairy equipment that is to be treated with chemicals is thoroughly cleaned the action is seriously interfered with. Prucha[32] found that chloramine T and sodium hypochlorite lost little or none of the chlorine when exposed for 30 minutes at temperatures ranging from 26.7° to 71.1° C. (80° to 160° F.) but that the addition of a small amount of milk promptly caused a reduction in the available chlorine, which increased with an increase in the temperature or in the amount of milk added. In the absence of organic matter the addition of one per cent sodium hydroxide to a sodium hypochlorite solution increased the stability but, in the presence of organic matter, the available chlorine disappeared faster with the sodium hydroxide added. This investigator also noted a slower destruction of *Escherichia coli* when small amounts of milk were added to solutions of various chlorine compounds than when the milk was absent.

There appears to be little need for the general use of chemicals in the treatment of dairy equipment. Steam is available in every plant of any considerable size and, if there is no steam in small plants or on farms, there should be an abundance of hot water. The proper washing of dairy equipment is impossible without hot water so that it constitutes one of the necessities in a dairy establishment. The use of a chemical solution in equipment without subsequent drying leaves a moisture supply in which the growth of organisms may occur on extended holding and there may also be rusting. It is necessary that the chemical solutions used be up to a definite strength, if they are to be effective, and with the deterioration that occurs with certain of the commercial preparations, this is not always maintained in practice. Traces of the chemical used may also be carried over into the dairy products unless rinsing is practiced.

The action of various chemicals under different conditions is by no means completely understood. Wade, Archibald and Whittaker[33] reported that, from their experiments, it appears a calcium hypochlorite solution containing available chlorine in amounts usually advised for treating equipment and utensils in milk plants, and even up to 500 parts per million, can not be depended on to destroy human tubercle bacilli. Chloramine, in a strength of 93 to 95 parts per million of available chlorine, for a period of three minutes was effective.

---

[32] Ice Cream Rev. 11. P. 86. Feb., 1928.

[33] Jr. Bact. 15. P. 189. 1928.

Chemical action of washing powders. It should be recognized that the washing powders used in dairy plants have a destructive action on organisms, especially when used in strong solutions and at washing temperatures. This action may be a factor in the value derived from washing powders in the cleaning of equipment and, in certain types of bottle washers, it is very important. Levine, Peterson and Buchanan [34] studied the germicidal action of sodium hydroxide, sodium carbonate and trisodium phosphate on dried bacterial spores at 69° to 70° C. (156.2° to 158° F.) and concluded that factors other than the hydrogen-ion concentration were involved. When each of the compounds studied was considered individually, the germicidal efficiency was a direct function of the hydrogen-ion concentration. These investigators [35] also reported results indicating that the presence of sodium carbonate enhanced the disinfecting powers of sodium hydroxide and suggested that the undissociated sodium hydroxide, rather than the hydroxyl-ion concentration, may be the controlling factor in disinfection with alkali. Levine, Toulouse and Buchanan [36] noted that the addition of sodium chloride, sodium carbonate, or trisodium phosphate to sodium hydroxide markedly decreased the killing time at 50° C. (122° F.) and 60° C. (140° F.); as the concentration of salts added to the sodium hydroxide was increased, the killing time was decreased but at a decreasing rate.

Mudge and Lawler [37] studied the action of alkali solutions on the bacteria found in milk bottles returned to a plant. The organisms were secured by shaking out dirty bottles with small amounts of sterile water, which were then collected in a container; the suspension of organisms thus obtained was exposed to various concentrations of alkali. The hydrogen-ion concentration was found to be an important factor in the germicidal action, as were also the time and temperature.

---

[34] Ind. and Eng. Chem. 19. P. 1338. 1927.

[35] Ind. and Eng. Chem. 20. P. 63. 1928.

[36] Ind. and Eng. Chem. 20. P. 179. 1928.

[37] Ind. and Eng. Chem. 20. P. 378. 1928.

CHAPTER V

GROWTH OF MICRO-ORGANISMS IN MILK AND CREAM

The numbers of micro-organisms in milk and cream, as a result of contamination from various sources, are often increased and sometimes greatly increased through multiplication during the holding period. It has been pointed out that even under very insanitary methods of production the bacterial content of milk at the time it leaves the stable only occasionally exceeds 500,000 per cc. and that the very high counts found in many samples on reaching the dairy plants or consumers are largely due to growth.

The rate at which organisms increase in milk is determined by two factors, the temperature of holding and the species present. The holding temperature also greatly influences the types of organisms that will grow since the maximum, optimum and minimum growth temperatures vary a great deal with different species and a temperature that is quite satisfactory for the growth of one may entirely prevent the development of another; even within a species there may be great variations in the way different cultures develop at a given temperature.

The germicidal action of milk. For a certain period after milk is drawn there may be a decrease in the number of organisms in it, as determined by the plate method, and this is ordinarily considered to be due to a germicidal property. The blood and body fluids in general have the ability to destroy many types of organisms and, accordingly, some such action might be expected with milk. The germicidal action of milk is, however, rather weak and perhaps should be looked upon as a restraining or antiseptic action rather than one actually destroying organisms. By adding pure cultures of bacteria to carefully drawn milk and determining the numbers after varying periods, very definite decreases are sometimes secured. Heinemann and Glenn[1] noted that, with this procedure, the inoculation of fairly large numbers of bacteria into cows' milk gave a more decided decrease than the inoculation of only small numbers. The germicidal action is of little, if any, practical importance in dealing with the problems of holding milk and cream, although at one time it seems to have been used as an excuse for careless methods when the holding period was to be short. Fokker,[2] in an investigation of goats' milk, was probably the first to note the germicidal action of milk; he observed slower souring and a smaller number of colonies on plates poured with inoculated raw milk than with heated milk from the same source similarly inoculated.

---

[1] Jr. Inf. Dis. 5. P. 534. 1908.

The decrease in the plate count on a lot of milk for a time after it is drawn can not be said to be pronounced and clear-cut and it is only through a considerable number of trials that such a decrease can be definitely established. There are, however, many results which show lower counts for subsequent determinations than for the first, and this is especially true of milk held at fairly low temperatures. The following counts, taken from the work of Rosenau and McCoy,[3] illustrate the changes in the numbers of bacteria in portions of a sample of milk, from a healthy cow, held at various temperatures.

Changes in the Numbers of Bacteria in the Milk of a Healthy Cow

Time	Bacteria per CC.		
	Room Temperature 16°–23° C. (60.8°–73.4° F.)	15° C. (59° F.)	37° C. (98.6° F.)
At once......	400	400	400
After 2 hours	430		
4	100	450	350
6	350	600	2,100
8	450	300	345,000
10	500	350	1,780,000
12	400	300	32,800,000
14	500	400	75,500,000
24	5,000	2,000	Sour
36	60,000	2,000	Sour

The germicidal action of milk is influenced to a large extent by the temperature of holding, as is indicated by the above data. At a comparatively high temperature, such as 37° C. (98.6° F.), the decrease in the count is complete in a few hours and a rapid increase then follows, while at lower temperatures a definite increase requires considerably longer periods. At 0° C. (32° F.) there may be a gradual decrease extending over a period of more than a week.

---

[2] Fortschr. der Med. 8. P. 7. 1890.

[3] U. S. Pub. Health Mar-Hosp. Serv. Hyg. Lab. Bul. 56. P. 457. 1909.

Early in the investigations of the germicidal property of milk Hunziker[4] pointed out that, "The degree of the germicidal action varies greatly in milk of different animals and sometimes in milk of the same animal taken at different milkings." The variation in the milk of individual animals has also been noted by Chambers,[5] who found that in a group of 11 cows the milk of only 3 possessed a germicidal action. Hanssen[6] found the property greatest in the summer time.

Different organisms are influenced very differently by the germicidal action of milk and, accordingly, this property must be considered as more or less specific. The extreme variation is illustrated by samples of milk which show a pronounced germicidal action for one organism and none at all for another. Organisms which grow very rapidly in milk appear to be included among the species influenced by the germicidal property. Heinemann and Glenn[7] found that *S. lactis* increases in milk from the beginning, although it may be inhibited to some extent, and Sherman and Curran[8] noted a slight but definite inhibitory effect on this organism. Chambers,[9] however, reported that in the milk of two cows, which was known to possess germicidal properties, there was no action on *S. lactis.*

Action of heat on the germicidal property. High temperatures destroy the germicidal property of milk but the temperature at which such destruction occurs seems to vary with different organisms. Rosenau and McCoy[10] found that the germicidal property for *A. aerogenes* was influenced at 55° C. (131° F.) for 30 minutes and almost destroyed at 60° C. (140° F.) for 20 minutes, while the action against *Eberthella typhosa* was not influenced at 60° C. for 20 minutes but materially influenced at 70° C. (158° F.) for 30 minutes. Freezing temperatures for short periods had no influence on the germicidal property, while freezing for long periods decreased it. Hanssen,[11] using *E. typhosa* and paratyphoid B, found that 75° C. (167° F.) for 15 minutes destroyed the bactericidal property while 70° C. for 15 minutes and 63° C. (145.4° F.) for 30 minutes did not. The earlier work of Hunziker,[12] which involved only the organisms gaining entrance to milk during the production, showed that heating to 65° C. (149° F.) for 40 minutes destroyed the germicidal property.

---

[4] N. Y. (Cornell) Agr. Expt. Sta. Bul. 197. 1901.

[5] Jr. Bact. 5. P. 527. 1920.

[6] British Jr. Expt. Path. 5. P. 271. 1924.

[7] See Ref. 1.

[8] Proc. Soc. Expt. Biol. and Med. 22. P. 15. 1924.

[9] See ref. 5.

[10] See ref. 3.

Hammer and Baker [13] noted that when butter cultures were prepared from milk pasteurized with different exposures, the milk heated to 63° C. (145.4° F.) for 30 minutes developed acid and coagulated more slowly than milk heated to considerably higher temperatures. Essentially the same rate of development of the cultures occurred after heating to 71° C. (159.8° F.) for 30 minutes as when a higher temperature was used for this period. With a heating time of 30 minutes the influence of the heat seemed to be gradual and to spread over a considerable temperature range instead of occurring completely at a definite temperature.

Causes of the decreases in bacteria in milk. The action of milk in causing a decrease in the number of organisms, as determined by the plate method, seems to be dependent on several factors. Certain bacteria apparently find conditions unfavorable and rapidly die out. Some of the leucocytes or white blood cells present may retain their ability to ingest bacteria after the milk is drawn, although there seems to be no very good evidence of this; the examination of milk sediments frequently shows leucocytes containing bacteria, but these may have been ingested before the milk left the udder. Certain of the bacteria gaining entrance to milk are apparently clumped by agglutinins and the bacterial count, as determined by the plate method, might thus be decreased since, if the organisms remain clumped, they would give rise to fewer colonies than if agglutination had not occurred. The agglutinins undoubtedly come from the blood of the producing animal and, since variations occur in the agglutinin content of blood, similar variations are to be expected in milk; this may be an important factor in accounting for variations in the germicidal property of different lots of milk. Clumps of organisms, some of which contain enormous numbers, are common in milk sediments and, while some of these are undoubtedly due to the manner of growth of the particular species, others may be due to agglutination. Chambers[14] found a clumping of certain organisms added to milk but no direct relationship between agglutination and germicidal action. It seems that antibodies other than agglutinins are also present in milk and that these may be a factor in the germicidal property of this product.

---

[11] See ref. 6.

[12] See ref. 4.

[13] Ia. Agr. Expt. Sta. Res. Bul. 106. 1928.

Hanssen[15] has proposed the theory that the bactericidal property of milk is in close relation to the oxidizing enzymes and has suggested that the variations in the bactericidal power found at different seasons depend upon variations in the feed of the cow through their influence on the content of oxidizing enzymes in the milk.

Bacterial changes at various temperatures. As already stated, the temperature at which milk is held not only determines the rate of bacterial growth but also has an influence on the types of organisms that develop and, accordingly, when milk is held at any given temperature it undergoes a certain change or combination of changes with more or less constancy.[16] In agreement with this, each of the various fermentations is ordinarily encountered under fairly definite temperature conditions. Often these are not the most favorable for the causative organisms, but, because of their pronounced restraining action on competing organisms, they are comparatively very favorable; for example, *Bact. viscosum* grows more rapidly at 21° C. (69.8° F.) than at 10° C. (50° F.) but at the former temperature *S. lactis* is so active that *Bact. viscosum* rarely, if ever, produces ropiness, while at the latter temperature it finds much less competition.

Milk held frozen. In milk held below the freezing point which, although varying somewhat with the composition, is approximately —0.55° C. (about 31° F.), there is no multiplication of bacteria. The methods by which bacteria secure their nutrients preclude growth as long as the milk is frozen throughout. Usually there is a tendency to a decrease in numbers, the rate being dependent on the resistance of the contained organisms to the unfavorable conditions. The following data, taken from the results reported by Ravenel, Hastings and Hammer,[17] illustrate the changes in the numbers of bacteria, as determined by the plate method, and in the acidity of milk held in a frozen condition; the temperature of holding was —9° C. (15.8° F.).

---

[14] See ref. 5.

[15] See ref. 6.

[16] Conn. (Storrs) Agr. Expt. Sta. 16th An. Rpt. P. 27. 1904.

[17] Jr. Inf. Dis. 7. P. 38. 1910.

Changes in Milk Held at − 9° C. (15.8° F.)

Age in Days	Bacteria per CC.	Acidity, Per Cent
0	130,000	0.16
6	108,500	0.15
15	95,500	0.16
20	92,000	0.15
36	100,500	0.14
74	127,000	0.13
106	63,750	0.11
160	51,000	0.11

The decrease in the acidity, which was small but quite definite because it was so gradual, is difficult of explanation. Eventually the milk developed an "off" flavor.

Hilliard and Davis [18] concluded that, with freezing temperatures, there is much less destruction of bacteria in milk and cream than in pure tap water, due no doubt to the physical protection offered the bacteria by the colloidal and solid matter in suspension. The degree of cold below freezing was not an important factor in the destruction of bacteria; crystallization, probably resulting in mechanical crushing, seemed to be important in causing death. The relationship of the mechanical protection from sheering ice crystals given by solutes to the viability of bacteria at low temperatures has also been pointed out by Sedgwick, Hamilton and Funk.[19]

Milk that has been frozen for any length of time does not regain its normal consistency on being thawed because the freezing at first largely involves the water and results in a concentration and clumping of the milk solids which makes them difficult to reincorporate without some special process. Cream intended for ice cream making is sometimes skimmed very rich during the surplus season and stored in a frozen condition for considerable periods; on thawing, it can readily be gotten into a satisfactory physical state by homogenization. The freezing and storage of milk on a commercial scale is not likely to prove profitable because the expense involved is so high, compared to the value of the product. There does not seem to be any danger in the use of milk or cream held in a frozen condition for considerable periods because the production of toxic products is not to be expected when there is no growth of micro-organisms; however, abnormal flavors, more or less pronounced, sometimes develop.

[18] Jr. Bact. 3. P. 423. 1918.

[19] Absts. Bact. 1. P. 49. 1917.

Milk held just above the freezing point. Milk and cream held just above the freezing point, for example, at 0° C. (32° F.), ordinarily show a slight decrease in the bacterial count and then, after from one to three weeks, multiplication begins and continues until very large numbers of organisms are present. The following data, also taken from the results of Ravenel, Hastings and Hammer,[20] show the changes in the numbers of bacteria, as determined by the plate method, and in the acidity of milk held at 0° C.

Changes in Milk Held at 0° C. (32° F.)

Age in Days	Bacteria per CC.	Acidity, Per Cent
0	130,000	0.16
6	72,500	0.15
15	633,500	0.17
20	3,230,000	0.17
36	34,950,000	0.21
74	91,500,000	0.54
106	39,750,000	0.65
160	32,650,000	0.92

In the milk on which these data were secured there was an abnormal flavor and a clumping of the casein after 36 days while after 106 days there was gas formation, a bad odor and a digested appearance. Chemical examinations at the end of the holding period showed that the total nitrogen had decreased, some apparently being carried away as free nitrogen in the gas that escaped, and that 72 per cent of the total nitrogen was in a soluble form, instead of the usual 20 to 25 per cent for normal milk. The development of acid was probably not due to the action of *S. lactis* but to organisms whose growth is much less restricted by low temperatures.

In milk held at from −1.1° to −0.6° C. (30° to 31° F.) Pennington[21] noted that, although the bacterial count was in the hundreds of millions per cubic centimeter, there was neither an odor or taste to indicate this and it was not until putrefaction was under way that the milk appeared objectionable. This investigator found that there were constantly present bacteria which formed acid, others which acted on the protein and still other neutral types; the acid formers were generally in relatively smaller numbers than in milk held at higher temperatures while the liquefiers were more numerous. Caseoses, amino acids and probably peptones increased in the milk, apparently at the expense of the casein. The rate of digestion varied with different samples, but at the expiration of two weeks was regularly pronounced.

---

[20] See ref. 17.

[21] Jr. Biol. Chem. 4. P. 353. 1908.

The protein decomposition of milk and cream is very objectionable, since it may result in the formation of toxic products, and the possibility of these being produced constitutes a definite danger when milk and cream are held for considerable periods at just above the freezing point. Certain of the cases of poisoning from ice cream have been very probably due to long storage of the cream used at approximately 0° C.; at higher temperatures bacterial changes would have occurred much more rapidly but would have involved primarily carbohydrate decomposition, which ordinarily does not yield harmful products, instead of protein decomposition.

Milk and cream are commonly stored at approximately 0° C. and such storage constitutes one of the very satisfactory methods of preserving these products. However, it should be recognized that even at this temperature the time of holding must not be excessive because of the possibility of the development of toxic products; it seems probable that at 0° C. ten days is about the limit of absolutely safe storage, although, of course, much longer periods have repeatedly been used without harmful results.

Milk held to 4.4° C. (40° F.). In milk and cream held at approximately 4.4° C. the changes that occur are, in a general way, essentially the same as those occurring at 0° C., although the increase in the number of organisms begins somewhat sooner. This indicates the possibility of the formation of toxic products at 4.4° C. with prolonged holding and also suggests that the period of safe storage is shorter than at 0° C.

Milk held at 10° C. (50° F.). At 10° C. the changes that take place in milk and cream are quite variable due to the fact that different types of organisms find conditions suitable for growth, while none of them are greatly favored. In some instances the first conspicuous change is the development of acid while, in others, abnormal fermentations occur. The bitter and ropy fermentations especially are likely to take place at temperatures approximating 10° C. because some of the organisms responsible for them grow fairly well and the competition from *S. lactis* is much less severe than at higher temperatures. The development of bitterness in milk and cream is most common during the colder months when the products are often held at about 10° C. in their ordinary storage on farms or in homes. Ropiness usually occurs in milk kept reasonably cold, rather than in milk held at higher temperatures. In general, it can be said that at 10° C. the type of change that will develop can not be predicted with as much certainty as at temperatures either above or below it.

Milk held at 21.1° C. (70° F.). At 21.1° C. milk and cream, with the usual bacterial flora, quickly develop lactic acid because of the very favorable conditions for the growth of *S. lactis.* Accompanying or following this, there is often gas formation, due to the action of Escherichia-Aerobacter forms or the lactose fermenting yeasts and the development of undesirable flavors and odors, as a result of the activity of a wide variety of organisms. In milk held at temperatures approximating 21.1° C. the conditions are so ideal for the growth of *S. lactis* that this type dominates the changes that occur and quickly excludes the organisms that do not tolerate considerable acid.

Milk held at 37° C. (98.6° F.). In milk held at 37° C. rapid acid production ordinarily occurs as a result of the growth of *S. lactis,* and gas production is especially prominent because of the very favorable conditions for the usual gas forming organisms. This temperature is also very suitable for the development of certain of the lactobacilli and, as a result of their activity, considerable acid may be developed; in the warmer sections of the United States individual lots of cream intended for the manufacture of butter sometimes contain 2 per cent or more acid. Milk and cream are, of course, not likely to encounter a temperature as high as 37° C., except in warm sections when there is little or no attempt at cooling.

Milk held above 37° C. (98.6° F.). Many organisms are capable of growth at temperatures above 37° C. It seems that certain types even grow fairly rapidly at the temperatures commonly used in the holding method of pasteurization; these organisms, in some instances, have been responsible for high counts in pasteurized milk, especially when pasteurization equipment has been used for one batch of milk after another without sterilization between runs, so that the organisms have become established in it. It should be recognized, however, that at temperatures not greatly above 37° C. the rather rapid destruction of organisms begins. Hammer[22] observed that in the double pasteurization of sour cream intended for buttermaking a considerable percentage of the organisms was killed by the preheating to 50° C. (122° F.).

---

[22] Ia. Agr. Expt. Sta. Bul. 156. 1914.

during warm weather without refrigeration, a reduction of only a few degrees is of no importance.

The data given further indicate that the rate of multiplication of the bacteria is influenced by the quality of the milk and that the increase in the organisms, on a percentage basis, is less in clean milk than in dirty milk. This is to be expected since, with the clean milk, a considerable portion of the organisms have come from the interior of the udder and, accordingly, are types that grow comparatively slowly while, with the dirty milk, many of the organisms have come from external sources and include types that multiply rapidly. This difference between clean and dirty milk explains why conditions that are reasonably satisfactory in preventing changes in certain lots of milk or cream are unsatisfactory for other lots and also why the conditions of production have a greater influence on the quality of a lot of milk or cream after a period of holding than might be expected from the actual number of bacteria introduced by the careless methods.

Holding temperatures for milk and cream. The holding of milk and cream for varying periods is necessary on practically all dairy farms and in various dairy plants. It is most satisfactorily carried out by entirely preventing the growth of organisms but this is not always possible because the low temperatures required are not available. The use of refrigeration machinery or ice is common in dairy plants and may be profitable on certain dairy farms, especially when the ice can be secured for the harvesting, but is not possible on many of the farms because the cost would be so high, compared to the value of the product cooled. Well and spring water are extensively advocated for cooling and, in many sections, make it possible to keep milk or cream in a satisfactory condition for a reasonable period. Farms supplying special quality milk, which commands a comparatively high price, often find it economically sound to provide good facilities for cooling and holding their product, even if the expense is considerable.

When morning milk and evening milk from the day before are delivered to milk plants in the morning, the evening milk often has a much higher bacterial count than the morning milk. This is illustrated by the following data secured at the Iowa Agricultural Experiment Station on milk supplied from various farms; results showing that the bacterial content of the evening milk can be kept low are also included.

From these results it is evident that growth at 4.4° C. is comparatively slow and that at 10° C. it is considerably more rapid, while the difference between 10° C. and 15.6° C. is still more striking. The following data also reported by Ayers, Cook and Clemmer compare the growth at 15.6° C. (60° F.) and 21.1° C. (70° F.):

CHANGES IN THE NUMBERS OF BACTERIA IN MILK AT 15.6° C. (60° F.) AND 21.1° C. (70° F.)

Clean Milk (Average 16 Samples)

Temperature of Holding	Bacteria per CC.			
	Fresh	12 Hours	24 Hours	48 Hours
15.6° C.	3,243	4,056	123,562	26,176,923
21.1° C.	3,243	19,312	10,006,875	2,014,692,307

Dirty Milk (Average 13 Samples)

Temperature of Holding	Bacteria per CC.			
	Fresh	12 Hours	24 hours	48 Hours
15.6° C.	707,761	3,376,961	48,550,923	296,100,000
21.1° C.	707,761	6,608,846	221,916,666	1,853,000,000

In these data also a difference of a few degrees resulted in a considerable difference in the rate of multiplication of the organisms.

The above tabulations indicate that the range from 10° C. to 21.1° C. is particularly important from the standpoint of the influence on the rate of multiplication of bacteria and, accordingly, on the time of the appearance of undesirable changes; they also suggest that, on the ordinary producing farms where this range is largely involved because of the use of well water for cooling, it is especially important that the cooling be as complete as possible and carried out quickly. At considerably higher temperatures a few degrees may make but little difference in the length of time milk and cream may be satisfactorily kept; for example, where these products are held under very unfavorable conditions, such as in the shipment of cream during warm weather without refrigeration, a reduction of only a few degrees is of no importance.

The data given further indicate that the rate of multiplication of the bacteria is influenced by the quality of the milk and that the increase in the organisms, on a percentage basis, is less in clean milk than in dirty milk. This is to be expected since, with the clean milk, a considerable portion of the organisms have come from the interior of the udder and, accordingly, are types that grow comparatively slowly while, with the dirty milk, many of the organisms have come from external sources and include types that multiply rapidly. This difference between clean and dirty milk explains why conditions that are reasonably satisfactory in preventing changes in certain lots of milk or cream are unsatisfactory for other lots and also why the conditions of production have a greater influence on the quality of a lot of milk or cream after a period of holding than might be expected from the actual number of bacteria introduced by the careless methods.

Holding temperatures for milk and cream. The holding of milk and cream for varying periods is necessary on practically all dairy farms and in various dairy plants. It is most satisfactorily carried out by entirely preventing the growth of organisms but this is not always possible because the low temperatures required are not available. The use of refrigeration machinery or ice is common in dairy plants and may be profitable on certain dairy farms, especially when the ice can be secured for the harvesting, but is not possible on many of the farms because the cost would be so high, compared to the value of the product cooled. Well and spring water are extensively advocated for cooling and, in many sections, make it possible to keep milk or cream in a satisfactory condition for a reasonable period. Farms supplying special quality milk, which commands a comparatively high price, often find it economically sound to provide good facilities for cooling and holding their product, even if the expense is considerable.

When morning milk and evening milk from the day before are delivered to milk plants in the morning, the evening milk often has a much higher bacterial count than the morning milk. This is illustrated by the following data secured at the Iowa Agricultural Experiment Station on milk supplied from various farms; results showing that the bacterial content of the evening milk can be kept low are also included.

Bacterial Counts on Morning and Evening Milk Delivered in the Morning

Producer	Date	Bacteria per CC.	
		Morning Milk, About 4 Hours Old	Milk from Evening before, About 16 Hours Old
A	Feb. 14	8,400	8,100
B	Mar. 28	9,300	29,000
C	Mar. 28	5,900	6,700
D	Mar. 29	45,000	310,000
A	Mar. 29	1,400	2,300
D	May 17	895,000	940,000
E	June 23	54,000	665,000
D	June 23	270,000	10,250,000
A	June 23	16,000	29,000
E	June 30	25,000	25,000
D	June 30	215,000	26,000,000
A	June 30	16,000	29,000
E	Aug. 11	7,700	985,000
D	Aug. 11	340,000	26,000,000
A	Aug. 11	30,000	50,000
B	Aug. 11	81,000	5,000,000
C	Aug. 11	50,000	2,550,000
D	Sept. 21	8,700	17,000 *
A	Sept. 21	4,500	4,100
E	Sept. 21	9,500	82,000

* Producer had been having milk returned just before this.

The comparatively high counts on many of the lots of evening milk are undoubtedly due to slow and incomplete cooling. In general, the actual contamination of the morning milk is greater than that of the evening milk because of the more unfavorable condition of the stable and cows and the less attention given the utensils following the evening milking, but this disadvantage is often more than counterbalanced by the multiplication of organisms during the night.

Dearstyne and Jones[26] found great variations in the bacterial contents of night and morning lots of milk supplied to various dealers and pointed out the importance of this in the grading of milk on the basis of the numbers of contained bacteria. Macy[27] studied, under summer conditions, the growth of bacteria in uncooled morning milk from the time of production until delivery at the pasteurizing plant and noted regular increases, although in the samples with a low initial count the final count was also low. He concluded that in areas where the conditions can be satisfactorily controlled the farmer might be permitted to deliver uncooled morning milk to the pasteurizing plant but that, as a general practice in all communities, it is not to be recommended.

---

[26] Jr. Dairy Sc. 2. P. 504. 1919.

[27] Jr. Dairy Sc. 5. P. 502. 1922.

In plants where ice or a refrigerated room is used for storage the temperature of the milk or cream should be gotten down to approximately 0° C. (32° F.) whenever the holding is to be for more than over night. The physical condition of these products will not be seriously influenced if the temperature is so low that there is slight freezing against the walls of the cans and, when cream is to be held for several days, as in plants manufacturing ice cream, it is a good practice to get the temperature so low that such freezing occurs. With bottled milk that is to be held for the short period between bottling and delivery the temperature should be reduced below 10° C. (50° F.) and preferably to 4.4° C. (40° F.) or even lower. Although for very short periods the multiplication of bacteria at 10° C. is negligible, especially with clean milk, lower temperatures should be used because they aid in getting the milk or cream to the consumers without undesirable temperatures being reached. Their value in the development of a desirable cream line is also important.

In the cooling of all dairy products for the purpose of holding, it is essential that the temperature reduction be accomplished as quickly as possible. Air conducts heat slowly and, accordingly, a can of uncooled milk or cream placed in a room at 0° C. (32° F.) may require hours before it reaches the temperature of the room. In dairy plants the actual cooling, when at all possible, should be carried out with coolers or vats and the refrigerated room looked upon only as a storage room and not as a place for cooling. Bottled milk put into coolers should be iced, unless it has been reduced to a temperature satisfactory for the prevention of the growth of organisms. On the producing farms where the cooling of milk or cream is accomplished by standing the cans in cold water, the contents of the cans should be occasionally stirred, especially during the early part of the cooling, so that the material at the center does not remain warm for extended periods.

CHAPTER VI

BODY CELLS IN MILK

Milk normally contains cells that come from the body of the producing animal. Some of them are from the linings of the teat canal, milk cistern and milk ducts and even from the surface of the teats and udder, but the majority are cells that, because of their general appearance, are commonly believed to be leucocytes, or white blood cells. These cells are largely of the polymorphonuclear type but other varieties also occur; occasionally cells containing bacteria are observed. Certain investigators, such as Hewlett, Villar and Revis,[1] consider that the cells so common in milk are not leucocytes. The main reasons for this appear to be that even in fresh milk the vast majority of the cells never stain like active leucocytes in blood and are not amoeboid. Erythrocytes, or red blood cells, are occasionally found in milk as a result of the rupture of a blood vessel in the udder or teat but are never confused with the cells normally found in milk because of the lack of a nucleus.

The body cells of milk require consideration in connection with the micro-organisms in this product because large numbers of cells are often associated with udder inflammations and the organisms responsible for these conditions are of significance from the standpoint of human disease. However, the relationship between abnormal udder conditions and the number of body cells is not a definite one and cell determinations alone admit of only very limited conclusions, although often valuable in connection with other evidence. Before the variations that may occur in the numbers of cells in normal milk were recognized, the milk from excellent dairies was occasionally excluded from certain cities because the cell counts were believed to indicate abnormal udder conditions.

Methods of determining body cells. Various methods have been used for the determination of the body cells in milk. The more important of these are the following:

---

[1] Jr. Hyg. 9. P. 271. 1919.

Trommsdorff method. The Trommsdorff method involves the use of a special centrifuge tube which is drawn out at the bottom to a fine bore and calibrated. Ten cubic centimeters of milk are whirled in this for 10 minutes in an ordinary centrifuge and the per cent of sediment then read off directly. The method gives no information as to what the material in the sediment consists of, so that in case a high percentage is found, a microscopic examination should be made.

Stewart-Slack method.[2] In the Stewart-Slack method two cubic centimeters of milk, in a tube closed at each end with a rubber stopper, are whirled in a special centrifuge. The stopper is removed from the cream end, the cream layer broken with a wire and the milk poured out. If the other stopper is then carefully removed, the sediment will adhere to it. The sediment is smeared over a definite area, usually four square centimeters, using a small amount of water to aid in the distribution, and the slide then quickly dried and stained with methylene blue. The preparation shows the body cells and also the bacteria that are stainable with the stain used. The average number of either of these per standard microscopic field can be determined and the results expressed directly or calculations can be made to a cubic centimeter basis, as in the direct microscopic method of determining the bacteria in milk. Many of the body cells and bacteria are carried up into the cream layer so that the results are more valuable on a comparative than on an absolute basis.

Doane-Buckley method.[3] The Doane-Buckley method consists of concentrating the body cells present in milk and then counting the number in a definite volume of the concentrate, by the use of a blood cell counting apparatus. Since the procedure was originally devised, the necessity of heating the milk to prevent the fat globule clusters from carrying up many of the cells has been shown by various investigators.

Ten cubic centimeters of milk are heated in a centrifuge tube to 70° C. (158° F.) for 10 minutes or 80° C. (176° F.) for one minute, after which the milk is cooled by standing the tube in cold water. Centrifuging is then carried out. The speed and time used have varied a great deal but should be ample to sediment as many of the cells as possible; 2,000 revolutions per minute for 20 minutes in an ordinary centrifuge is satisfactory. The fat is removed with a cotton swab and the skim milk aspirated down to about the one cubic centimeter mark. Water is then added up to the 10 cubic centimeter mark, the sediment thoroughly distributed and centrifuging again carried out, using one-half of the original period. The liquid is next aspirated down exactly to the one cubic centimeter mark, the sediment thoroughly mixed through the liquid and counts made with a blood cell counting apparatus. The calculations are made so that the results are expressed on the cubic centimeter basis. In some instances a stain has been added to the material in the centrifuge tube to make the counting easier but, with a proper adjustment of the light, the cells can be clearly seen when they are unstained.

---

[2] Standard Methods of Milk Analysis. Pub. by Am. Pub. Health Assn. 5th ed. P. 28. 1927.

[3] Md. Agr. Expt. Sta. Bul. 102. 1905.

Savage [4] has also devised a method for counting the leucocytes in milk by means of a blood cell counting apparatus.

Direct method. The method used for the direct enumeration of the bacteria in milk is readily applicable to the counting of the body cells.

Comparison of various methods. The Doane-Buckley method of determining the numbers of cells in milk appears to be especially valuable from the standpoint of the agreement of duplicates. With 23 samples Russell and Hoffmann [5] found that the variations in duplicate determinations ranged from 0 to 20.0 per cent and averaged 5.6 per cent when the Doane-Buckley method was employed, while with 20 samples a smeared sediment method showed variations in duplicates from 0.3 to 587.0 per cent and an average of 112.0 per cent. Campbell [6] employed the Doane-Buckley method with a number of washings of the sediment and found that, with 24 samples, the variations between duplicates ranged from 1.2 to 12.5 and averaged 4.2 per cent; without heating, however, the variations ranged from 0.6 to 78.1 and averaged 27.1 per cent. Ross [7] noted an average variation between duplicates of 12.76 per cent when 56 samples were examined with the Doane-Buckley method.

Numbers of cells in normal milk. Wide variations occur in the cell content of normal milk and these include not only variations between lots of milk from different sources but also variations from day to day in the milk from the same animal or herd. Ross,[8] using the Doane-Buckley method, found that the cell content of the milk from 50 cows that appeared normal varied from 4,000 to 3,576,000 per cc. Breed[9] employed the direct microscopic method and reported that, of 122 cows whose milk was examined, 59 gave cell counts under 500,000 per cc., 36 gave counts between 500,000 and 1,000,000 while 27 gave counts over 1,000,000; the average cell count was 868,000 per cc. With the herd maintained at the New York Agricultural Experiment Station, Breed found that 18 of the 25 cows gave average cell counts of less than 500,000 per cc., 6 gave counts averaging between 500,000 and 1,000,000 and 1 gave a cell count higher than 1,000,000. The 7 cows with counts above 500,000 per cc. included 1 that had suffered from udder trouble, 2 that had recently aborted and 2 old cows. The group of cows giving a low cell count included 1 with recent udder trouble that still had a hard lump in her udder, 1 that had recently aborted and 1 old cow. With a herd of 53 Guernseys maintained under excellent conditions, the average cell count was 895,000 per cc. and the individual counts varied from 65,000 to 6,950,000; 27 animals gave counts of less than 500,000 per cc., 16 gave counts between 500,000 and 1,000,000 while 10 gave counts over 1,000,000. Breed also reported the cell content of the milk of 11 goats, including 2 that had been studied by Breed and Stidger. The average for the 11 animals was 7,465,000 per cc. with the individual counts varying from 425,000 to 30,250,000.

---

[4] Jr. Hyg. 6. P. 123. 1906.

[5] Wis. Agr. Expt. Sta. 24th An. Rpt. P. 231. 1907.

[6] U. S. D. A. B. A. I. Bul. 117. 1909.

[7] N. Y. (Cornell) Agr. Expt. Sta. Bul. 303. 1911.

[8] See ref. 7.

Variations in milk from different quarters. A pronounced variation occurs in the cell content of milk from different quarters. The early results obtained by Doane,[10] before the Doane-Buckley method was modified, show this; some of the data are as follows:

Cell Content of Milk from Different Quarters

Cow Number	Cells per CC.			
	Left Front Quarter	Left Hind Quarter	Right Front Quarter	Right Hind Quarter
69	9,000	2,000	5,000	77,000
83	15,000	8,000	4,000	14,000
39	25,000	275,000	29,000	96,000
39 (later)	31,000	800,000	45,000	49,000

Variations in milk from different portions of the milking. The different portions of a milking show variations in the cell counts that are often pronounced. In general, the strippings run considerably higher than the other portions of the milking, while the fore milk may be either higher or lower than the middle milk. The following data, selected from those obtained by Ross,[11] illustrate the relationship:

---

[9] N. Y. (Geneva) Agr. Expt. Sta. Bul. 380, 1914.

[10] See ref. 3.

Cell Content of Fore Milk, Middle Milk and Strippings

Sample Number	Cells per CC. in			Per cent Increase per CC. of Strippings Over	
	Fore Milk	Middle Milk	Strippings	Fore Milk	Middle Milk
1	487,000	448,000	875,000	44.34	48.80
2	41,000	43,000	61,000	32.78	29.50
4	2,696,000	2,045,000	2,746,000	18.20	25.52
5	9,000	15,000	29,000	68.96	48.27
10	1,404,000	824,000	3,064,000	54.17	73.10
14	229,000	126,000	248,000	7.66	49.19
	Average increase 15 comparisons........			53.85	52.14

Russell and Hoffmann[12] studied 18 animals and found that the strippings contained, on an average, three times as many cells as the fore or middle milk. In the results reported by Campbell,[13] the strippings usually showed the highest cell count, although occasionally the fore milk was high.

The higher count obtained with the strippings than with the other portions of the milking may be due to a tendency of the manipulation of the udder during the stripping process to dislodge cells so that they can be more easily carried out with the milk. By vigorously manipulating the udder for five minutes before milking, Ross[14] was unable to consistently get an increase in the cell content of the milk over that of the two previous milkings. It is entirely possible, however, that the manipulation of the udder at the end of the milking period might have quite a different effect than manipulation before the milking was begun.

Comparison of evening and morning milk. Ross[15] compared the cell content of evening and morning milk. The average figures for nine cows were 435,778 per cc. for the evening milk and 342,333 for the morning milk, a difference of 93,445; however, the evening milk was the higher with only five of the animals. With three other animals studied over a long period, Ross found the average cell content higher in the evening milk than in the morning milk in the case of two of them. It seems probable that there is no very definite relationship between the cell content of milk and the time of the day it is drawn.

---

[11] See ref. 7.

[12] See ref. 5.

[13] See ref. 6.

[14] See ref. 7.

[15] See ref. 7.

Comparison of cell content and the per cent fat. In data secured by Ross[16] there is no evidence of any relationship between the cell content of milk and the per cent fat; results on three cows studied over a considerable period may be summarized as follows:

CELL CONTENT AND PER CENT FAT IN EVENING AND MORNING MILK

Cow Number	Evening Milk		Morning Milk	
	Average Cell Content per CC.	Average Per Cent Fat	Average Cell Content per CC.	Average Per Cent Fat
1	82,214	6.05	63,500	5.52
2	118,682	3.70	119,341	3.80
3	693,267	3.97	495,267	4.18

Influence of the stage of lactation. Colostrum milk quite regularly has a higher cell content than the normal milk secreted later. The following data, taken from results given by Ross,[17] illustrate this:

CELL CONTENT OF COLOSTRUM AND NORMAL MILK

Sample Number	Cells per CC.	
	Colostrum Milk	Normal Milk
1	713,500	18,000
2	3,935,000	13,000
3	332,000	20,000
4	500,000	58,000

---

[16] See ref. 7.

[17] See ref. 7.

Ross states that, "Apparently most of the cells present in colostrum are broken-down epithelial cells," while Breed[18] reports that, "No cells of a different type than those formed in later stages of lactation were found in the colostral milk." Breed tabulated, according to the month of lactation, the results of 246 determinations of the cell content of milk from 126 different animals of various breeds. The average cell counts obtained from the milk of animals near the end of the lactation period did not seem to be markedly higher than those for the earlier months. However, several very high counts were obtained from animals advanced in lactation and the evidence indicates that high counts are more common then than during the height of lactation.

Influence of breed and age. There appears to be no evidence that the breed of an animal has any influence on the cell content of the milk. The results of Russell and Hoffmann[19] showed a marked tendency for the cell content of milk to increase with an increase in the age of the cow. This may be due to a greater opportunity for the development of abnormal udder conditions with the animals that have been through a larger number of lactation periods.

Comparison of cell content of machine and hand drawn milk. In a comparison of the cell content of machine drawn and hand drawn milk, Breed[20] found that the average of 113 machine drawn samples was 309,000 per cc. and the average of 56 hand drawn samples was 381,000. These results indicate that the use of a milking machine does not tend to cause an excessive discharge of cells from the interior of the udder.

Influence of the condition of the udder. An infection of the udder is the most important factor causing a high cell content in milk. While high counts are sometimes obtained on milk from udders that are apparently normal, these are ordinarily somewhat less than the excessive counts obtained on milk from many infected udders. Certain abnormal udder conditions do not give a high cell count, however, and the numbers may actually be very low. There is no cell standard that can be used as a basis for determining milk from abnormal udders because of the comparatively high counts with certain lots of normal milk and the low counts with some abnormal lots. The mixing of the milk from different animals also complicates the problem through the dilution of the abnormal portions. In certain instances, especially with milk from only one animal, the cell counts may be so excessive that an abnormal condition is quite clearly indicated; with these excessive counts the volume of sediment obtained on centrifuging is generally large and it often has a yellowish appearance suggestive of pus. Information on the numbers and types of organisms present is very valuable in interpreting the results of cell determinations. One of the streptococci that is often the cause of udder infections appears in the milk in more or less characteristic chains so that, under certain conditions, the experienced analyst may easily recognize it, especially when it is accompanied by a high cell count. However, some of the organisms responsible for udder infections show nothing typical morphologically and, moreover, they may be present in the milk in only small numbers.

---

[18] See ref. 9. [19] See ref. 5. [20] See ref. 9.

Ross[21] has reported an instance in which a cow developed an abnormal quarter and the milk from this had a cell count of 62,400,000 per cc., while that from the other three quarters showed only 407,000. The highest cell count observed in milk at the Iowa Agricultural Experiment Station is 8,400,000; it was secured on milk that was evidently abnormal and a report from the owner of the cow showed that the milk had been abnormal for some time. The milk had been used over an extended period for the manufacture of butter and the butter consumed without any known harmful effect.

Russell and Hoffmann[22] have reported the results of cell counts on milk from three cows, each of which had an abnormal udder during part of the examination period. The highest cell count obtained with milk that showed evidence of garget was 3,625,000 per cc. and the lowest was 489,000.

Hoffmann[23] found that the injection of such inert materials as physiological salt solution, boric acid solution and distilled water produced a rapid and marked leucocytosis, as shown by the swelling and fever of the quarters injected and by the increased leucocyte content of the milk. A considerable number of the samples of milk showing high cell counts were reported as abnormal, but abnormal milk also gave counts that could not be considered excessive and it seems that there is no close relationship between the leucocyte content and an abnormal condition in the milk. The highest count reported by Hoffmann in the work on experimental leucocytosis was 25,400,000 per cc. and this was secured on abnormal milk coming from a quarter that had been injected the day previously with 300 cc. of sterile distilled water. The next highest count observed was 18,250,000 per

[21] See ref. 7.

[22] See ref. 5.

[23] Wis. Agr. Expt. Sta. Res. Bul. 6. 1909.

cc. and was obtained on abnormal milk coming from a quarter injected the day previously with 150 cc. of sterile 2 per cent boric acid solution.

Influence of clarification and separation. The clarifier removes a portion of the cells present in milk, as will be pointed out in the consideration of clarification. This effect must be kept in mind whenever cell counts are made on milk that has been handled in a plant since the number of cells originally present may have been materially reduced.

Separation also results in the removal of large numbers of cells. Occasionally a farmer reports that the amount of slime collecting in the separator bowl is suddenly increased; most often this is due to an excessive number of cells in the milk, as a result of one or more udder infections.

Cellular test for pasteurized milk. A method, for the determination of whether or not milk has been pasteurized, that involves the staining of the cells present in the milk has been devised by Frost.[24] The procedure consists of mixing the milk with an equal quantity of a special methylene blue stain, allowing the mixture to stand for at least 10 minutes and then centrifuging; the sediment is spread on a slide, air dried and examined microscopically, first under the low power and then with the oil immersion. The size and appearance of the leucocytes and the general character of the background are used in determining whether or not the milk has been heated. The stain employed is made by adding 1.5 grams of methylene blue powder to 1,000 cc. distilled water, allowing it to stand at 37° C. (98.6° F.) for several hours and then filtering. A tube closed at each end with a rubber stopper constitutes a centrifuge tube permitting the easy removal of the sediment.

Frost [25] has given the following summary of the appearance of the microscopic picture obtained with milk heated to 62.8° C. (145° F.) or above and held for 20 minutes or more: "The nuclei of practically all of the leucocytes are in compact masses. They are well stained. The background is lighter than the nuclei except in the thickest places. The leucocytes are usually surrounded by a 'dark halo.' They are small—not more than 7.5 microns in diameter. If the sample is examined soon after pasteurization or has been kept under conditions which prevent the growth of bacteria, the bacteria will not stain at all or at least variably or indistinctly." Slides prepared with raw milk show a background that is quite blue in the thicker places and the unstained leucocytes appear as clear areas in it.

---

[24] J. A. M. A. 64. P. 821. 1915.

[25] Univ. of Wis. Studies in Sc. No. 2. P. 151. 1921.

Frost and Moore[26] applied the cellular test for pasteurized milk to 260 of the routine milk samples analyzed by Chicago Health Department laboratories during July, 1918. All of 47 samples of raw milk were correctly placed although 11 showed indications of having been partially heated. Of the 213 samples of pasteurized milk, 26 seemed to have been insufficiently heated and 14 of these could be condemned by the high bacterial count; this leaves 12, or 4 per cent, of the samples unsatisfactory in spite of the fact that they were supposed to have been pasteurized and to have a low bacterial count.

Traum[27] also used a method of staining the cells with methylene blue to differentiate between raw and heated milk; the milk was centrifuged and the sediment smeared out and fixed before staining.

[26] Jr. Dairy Sci. 2. P. 189 1919.

[27] Am. Jr. Vet. Med. 10. P. 703. 1915.

CHAPTER VII

SPREAD OF DISEASES THROUGH MILK AND ITS DERIVATIVES

The prevention of the spread of diseases through milk and its derivatives constitutes the most important problem involving the bacteriology of these products. The prime consideration with any food material is its safety and when a product is used that is known occasionally to contain pathogenic organisms its bacteriological condition is of special concern. Many of the present practices in the preparation and handling of dairy products are the result of attempts to prevent the dissemination of pathogens through these materials and the improvements that are continually being made are often aimed primarily at increased safety.

Historical. The first important evidence of the spread of disease through milk appears to have been secured by Dr. Michael Taylor[1] of Penrith, England, in 1857. It should be noted that this was before the science of bacteriology had been developed and before any organism had been definitely proven the cause of a disease. Taylor observed a number of cases of typhoid fever that had only a milk supply in common. In September, 1857, a servant girl had returned to her family in Penrith suffering from typhoid fever. She lived with her parents and four other children in a cottage consisting of two poorly ventilated and poorly lighted rooms. The father kept three cows and sold milk to about 14 families. The mother milked the cows and distributed the milk from the kitchen. Two of the other children in the family developed the disease and the mother nursed all three patients, while also tending to the milk business. As soon as the servant girl was convalescent she helped with the distribution of the milk. In October and November, 13 cases of typhoid occurred among seven families securing milk from the cottage and from these primary cases a large number of others developed.

---

[1] Swithinbank and Newman. Bacteriology of Milk. P. 260. 1903. Orig. Edin. Med. Jr. P. 993. 1858.

Taylor concluded that the milk was contaminated in the kitchen from the typhoid patients being cared for there.

Apparently little immediate attention was given to the important possibilities suggested by the observations of Taylor, but this is not at all surprising when the extent of the bacteriological knowledge then available is considered. In 1867, an outbreak of scarlet fever occurred in Penrith and was also studied by Taylor.[2] Among a few scarlet fever cases there was a child that died after an illness of two or three weeks. In the cottage in which the child was nursed a small milk business was carried on. The milk was taken from the barn to the kitchen where it was strained and distributed to about 14 families. Fifteen cases of scarlet fever occurred in six of the families supplied and there was also a number of secondary cases. The inquiries which Taylor made led to the conclusion that the disease was spread through milk as a result of its infection in the cottage.

The evidence that man may acquire infectious material from diseased cows through the consumption of milk developed along a somewhat different line. In 1865, Villemin proved experimentally the infectious nature of tuberculosis by inoculating tuberculous material from man into rabbits and then soon afterward found that tuberculous material from cows produced a disease in rabbits identical with that from human sources. The inoculability of tuberculosis and the relationship of the disease in man and cattle suggested its spread through milk and this was later proved by experiments in which various investigators succeeded in producing the disease in animals by feeding milk from tuberculous cows.

The pioneer investigations showing that diseases may be spread through milk have been extended until hundreds of cases of various diseases have been definitely traced to milk supplies. Such instances have been reported from all the important countries of the world and the evidence showing the relationship of milk to the cases has been secured by many different observers. With certain diseases, milk has been incriminated so frequently that it demands serious consideration in any attempt at their control, especially since the cases due to infected milk may be largely prevented through proper methods of production, handling, and processing.

Importance of dairy products other than milk in spreading diseases. The spread of diseases through milk suggests that products derived from it may also be of importance in this connection, although the manufacturing methods involved may have a significant effect on the organisms present.

---

[2] See ref. 1. orig. Brit. Med. Jr. 2. P. 623. 1870.

Butter has been the cause of only a few recorded outbreaks of disease; this may be due to the retention in the product of a comparatively small percentage of the organisms in the cream, but the destructive action of the acid in the cream on certain of the pathogens appears to be a factor also. In the ripened cheeses some of the disease producing organisms apparently find it difficult to survive the conditions encountered during the ripening processes but cheese-borne epidemics occasionally occur. Ice cream has frequently been involved in the spread of diseases and it is evident that the freezing and holding temperatures do not result in the complete destruction of pathogens that may be present, although some of them are probably killed.

It should be noted that the extended period of holding with much of the butter and ripened cheese and the wide distribution of these products, because of the ease with which they can be kept, make the tracing of cases of disease to them more difficult than with milk or ice cream. This may be a factor in explaining the comparatively few instances in which they have been found involved.

Spread of diseases among farm animals. Infected milk and milk derivatives are also of importance in the spread of diseases among farm animals and, in this way, have been responsible for serious economic losses. Some of the early pasteurization regulations applied only to milk that was to be returned to farms for feeding purposes and were intended to prevent the spread of important animal diseases, especially tuberculosis.

Groups of diseases spread through milk. The diseases that are spread through milk fall into two groups as follows: (1) those in which the infectious material comes primarily from the producing animals and (2) those in which the infectious material comes primarily from human sources, either directly or by being carried in water, on utensils, etc. The first group contains diseases affecting both man and farm animals and one or more apparently affecting only farm animals, while the second group contains the diseases which, for the most part, naturally affect only man. The diseases of the second group often appear in epidemic form.

GROUP 1. DISEASES IN WHICH THE INFECTIOUS MATERIAL COMES PRIMARILY FROM THE PRODUCING ANIMALS

TUBERCULOSIS

The danger, to man, of milk from tuberculous cows was suggested by the results of a number of the early investigators. In 1882, Koch [3] reported the discovery of the organism causing tuberculosis and then, after extensive studies, concluded that bovine and human tuberculosis are identical. The results of Smith, published in 1896 [4] and 1898,[5] showed, however, that there are important differences between the organism commonly causing the disease in man and the one responsible for the disease in cattle and laid the foundation for a differentiation into the human and bovine types. The work of Smith at once raised the question of the importance of the bovine type of *Mycobacterium tuberculosis* as a cause of tuberculosis in man. At the British Congress on Tuberculosis in 1901, Ravenel presented evidence indicating that the bovine type affects man while Koch reported that, on the basis of further experimental work, he no longer believed that the bovine type was transmissible to man. The controversy thus begun led to extensive investigations in many different countries and these have shown definitely that man may contract tuberculosis from cattle in various ways.

Importance of the bovine type in human tuberculosis. The importance of the bovine type of *M. tuberculosis* in human tuberculosis has been investigated by isolating the organisms from cases and then determining the type. The work of Park and Krumwiede [6] in New York City along this line is especially important because of the number of cases studied (478) and also because they were unselected; the findings, which have been divided on the basis of the ages of the infected persons, are summarized in the table on page 157. From these data it is evident that the bovine type of *M. tuberculosis* is of little importance in adults but causes a considerable percentage of the tuberculosis in children. The high percentage of infections with this type in children under five years of age is of special interest.

Park and Krumwiede [7] have tabulated the results secured in vari-

---

[3] Berl. Klin. Wchnschr. 19. P. 221. 1882.

[4] Trans. Assoc. Am. Phy. 11. P. 75. 1896.

[5] Jr. Expt. Med. 3. P. 451. 1898.

[6] Jr. Med. Res. n. s. 20. P. 313. 1911.

[7] Jr. Med. Res. n. s. 22. P. 109. 1912.

BOVINE TYPE OF *M. Tuberculosis* IN HUMAN TUBERCULOSIS
(Results of Park and Krumwiede)

	Adults (Over 16 Years)		5 to 16 Years		Under 5 Years		Total		Double Infections
	Human Type	Bovine Type	Human Type	Bovine Type	Human Type	Bovine Type	Human Type	Bovine Type	
Number of cases....	305	1	46	9	91	25	442	35	1
Per cent bovine..		0.33		16.36		21.55		7.34	

ous investigations along this line, including their own. It should be noted that some of the studies involved only a few cases and that these were often chosen because of the probability of the bovine type being present so that the percentages are not entirely representative. The combined results are as follows:

BOVINE TYPE OF *M. Tuberculosis* IN HUMAN TUBERCULOSIS
Results of various investigators

	Adults (Over 16 Years)		5 to 16 Years		Under 5 Years		Total		Double Infections
	Human type	Bovine type	Human type	Bovine type	Human type	Bovine type	Human type	Bovine type	
Number of cases....	940	15	131	46	292	76	1363	137	11
Per cent bovine..		1.57		25.99		20.65		9.13	

Many of the cases of tuberculosis in children that have been studied as to the type of organism responsible have not been fatal cases but those yielding tuberculous material through operations or through autopsies following death from some other cause. Park and Krumwiede[8] found that, of their fatal cases under five years of age, 12.5 per cent were due to the bovine type; they have also compiled, from the results of various investigators, the following data dealing with fatal cases:

[8] See ref. 7.

BOVINE TYPE OF *M. Tuberculosis* IN FATAL TUBERCULOSIS IN CHILDREN
(Results of various investigators)

	5 to 16 Years		Under 5 Years	
	Human Type	Bovine Type	Human Type	Bovine Type
Number of cases	22	8	192	45
Per cent bovine		26.67		18.99

The importance of the bovine type in human tuberculosis varies with the location of the lesions. From the data which they compiled from various sources, Park and Krumwiede[9] have prepared the following table illustrating this:

PERCENTAGE INCIDENCE OF BOVINE TYPE OF *M. Tuberculosis*
(Results of various investigators)

Diagnosis	Adults (Over 16 Years), per cent	5 to 16 Years, per cent	Under 5 Years, per cent
Pulmonary tuberculosis	0.4	0.0	2.8
Tuberculous adenitis, cervical	2.7	38.0	61.0
Abdominal tuberculosis	20.0	53.0	58.0
Generalized tuberculosis, alimentary origin	14.0	57.0	47.0
Generalized tuberculosis	0.0	16.0	8.6
Generalized tuberculosis including meninges, alimentary origin	0.0	0.0	66.0
Tuberculous meningitis	0.0	0.0	4.6
Tuberculosis of bones and joints	3.3	6.8	0.0
Tuberculosis of skin	23.0	60.0	0.0

The British Royal Commission on Tuberculosis[10] found, in a study of 29 cases of primary abdominal tuberculosis, that 14 were due to the bovine type, 13 to the human type and 2 were mixed infections. The commission also investigated two groups of pulmonary cases;

---

[9] See ref. 7.

[10] Final Rpt. Great Britain Royal Com. on Human and Animal Tuberculosis. 1. Sec. 3. P. 9. 1911.

with the first group of 14 cases, the material came from post-mortem examination and the human type was found in all, while with the second group of 28 cases the material investigated was sputum and 26 were due to the human type while 2 were due to the bovine type.

Although the percentage of the total cases of tuberculosis due to the bovine type of *M. tuberculosis* is not large, being 7.34 per cent according to the data secured by Park and Krumwiede, the frequency with which the disease occurs makes this percentage of a great deal of importance. Moreover, the cases of tuberculosis in man due to the bovine type can, in all probability, be prevented more easily than those due to the human type since the infection with the former must be largely through dairy products and these can be rendered safe by proper methods. The elimination of tuberculous cows from many herds and the extensive use of pasteurization, especially in the larger cities, have undoubtedly done much to prevent infections through milk and its derivatives.

M. tuberculosis in milk. Soon after *M. tuberculosis* was isolated the examination of various lots of milk for its presence began. The first important investigation in the United States appears to have been reported in 1890 by Ernst.[11] In 1911, Briscoe and MacNeal [12] compiled data, from various countries, that had been reported in the literature and found that, of the 7,573 samples of milk for which results were available, 515 or 6.8 per cent were positive; 176 of the samples were examined microscopically which would make the results on this small portion open to question, Another compilation by Tonney, White and Danforth,[13] in 1927, included 16,700 samples of which 1,448 or 8.66 per cent were positive. The more important data from the United States are summarized in the table on page 160. These results show a great deal of variation in the percentages of the samples infected but it is evident from them that, during the period covered, *M. tuberculosis* was commonly present in the raw milk in a number of the important cities in the United States. It should be noted that the samples from New Haven, examined by Bartlett,[14] were from mixing tanks holding from 300 to 1,200 quarts each so that the milk from a considerable number of cows was involved in each examination. Tonney, White and Danforth estimated on the

[11] Mass. Agr. Expt. Sta. Bul. 8. P. 13. 1890.

[12] Ill. Agr. Expt. Sta. Bul. 149. 1911.

[13] Am. Jr. Pub. Health. 17. P. 491. 1927.

[14] Am. Jr. Pub. Health. 13. P. 807. 1923.

INCIDENCE OF *M. Tuberculosis* IN MILK

Investigator	City	Year	Type of Milk	Number Samples Examined	Number Samples Positive	Per cent Positive
Anderson	Washington	1908	raw	232	16	6.9
Mohler	Washington	1908	raw	73	2	2.7
Schroeder	Washington	1908	raw	36	2	5.5
Stokes	Baltimore	1907–9	raw	88	4	4.5
Hess	New York	1909	raw	107	17	15.9
Campbell	Philadelphia	1909	past.	8	1	12.5
			raw	123	18	14.6
Goler	Rochester	1909	past.	12	1	8.3
			raw	80	5	6.3
Tonney	Chicago	1910	raw	95	10	10.5
Bartlett	New Haven	1923	raw	41	18	44.0
Tonney, White and Danforth	Chicago	1923–5	raw	258	9	3.5

basis of their findings that during the three years prior to January 1, 1926, approximately 43,750 quarts of infected milk were sent each day to the plants pasteurizing milk for the Chicago market.

The rather common presence of *M. tuberculosis* in milk is also evident from other investigations. The Baltimore Health Department[15] examined sediments from milk from 175 dairy farms supplying that city and reported that the milk from 7 (4%) of the farms contained the organism of tuberculosis. Mohler and Washburn,[16] in a study of tuberculosis in hogs, examined 15 samples of separator slime from widely distributed sections and found 5 (33.3%) containing this organism.

In many instances the detection of *M. tuberculosis* in milk has not been followed by an investigation of the type. Tonney, White and Danforth,[17] however, studied the virulence for rabbits of the organisms secured from their positive samples and found them all to be of the bovine type.

The European results, both from Great Britain and the continent, also indicate the common presence of *M. tuberculosis* in milk. Some of the investigations involved large numbers of samples and showed essentially the same positive percentages as the studies in the United States.

[15] Div. of Bact., Health Dept., Baltimore. An. Rept. 1914. P. 36.

[16] U. S. D. A. Farmers' Bul. 781. Revised 1923.

[17] See ref. 13.

Examination of milk for M. tuberculosis. The examination of milk and other dairy products for *M. tuberculosis* involves the inoculation of experimental animals, usually guinea pigs. Microscopic studies are of little value because acid fast organisms other than *M. tuberculosis* may be present as a result of contamination from various sources. Small quantities of milk can be inoculated, but it is more satisfactory to centrifuge larger amounts and use the cream and sediment only. A mixture of these may be inoculated directly or may be treated with some such reagent as antiformin or an aqueous solution of sodium hydroxide to destroy many of the organisms that might be responsible for infections causing rapid death. The inoculations may be made subcutaneously, intraperitoneally or into the thigh muscles; subcutaneous inoculations result in a smaller number of deaths from acute infections among the animals but may require longer to produce the disease. It is advisable to inoculate several animals with each sample and more than one method of inoculation may be employed.

Tuberculosis in the experimental animals results in a loss of weight and characteristic lesions in various parts of the body, especially the liver and spleen. Microscopic examinations ordinarily show acid fast organisms in these lesions but they may be very few. In tuberculous guinea pigs the injection of one or two cubic centimeters of tuberculin commonly gives such a severe reaction that the animals die in a short time. Unless an animal appears diseased, the tuberculin is not usually injected until six or eight weeks following inoculation because of the time required for the disease to develop. If an isolation is to be attempted the animal should be killed when visibly diseased and tissues removed at once and suitable cultures made.

In an examination of a milk supply for the presence of *M. tuberculosis* one negative result means very little, because of the intermittent infection of the milk, and several examinations extending over a considerable period are advisable. Moreover, the absence of the organism in a milk supply at one time means nothing, as far as the future is concerned, and when tuberculosis is present in the producing animals the milk may suddenly contain the organisms. If raw milk free from *M. tuberculosis* is desired, it can only be assured by keeping the cow or herd free from the disease.

Methods by which milk from tuberculous cows is infected with M. tuberculosis. The infection of milk from tuberculous cows with *M. tuberculosis* is greatly influenced by the location of the lesions and their general character.

When there is a lesion in the udder the milk is very likely to contain the causative organism. Briscoe and MacNeal[18] tabulated data reported in the literature and found that of 39 samples, from visibly diseased udders, 34 or 87.2 per cent were positive; most of the investigators reported results on only a very small number of samples, due no doubt to the infrequency of this condition. In some of the studies on milk from individual cows the only samples found to contain *M. tuberculosis* were those from tuberculous udders. For example, Moore[19] noted in an examination of 87 samples of milk from individual animals that the 2 positive samples came from cows having udder infections. Milk from tuberculous udders often contains such large numbers of *M. tuberculosis* that many organisms can be seen in each field of a microscopic preparation made with the sediment and the milk is highly infectious for experimental animals.

Some of the tuberculous cows without infected udders give milk containing *M. tuberculosis* while others do not. Briscoe and MacNeal[20] compiled the data reported in the literature on the presence of the organism in the milk of tuberculous cows having no recognizable udder lesions and found that, of the 748 cows studied, the milk of 131 or 17.5 per cent was positive. Some of the samples of milk were carefully drawn while, with others, there was the possibility of infection after the milk left the udder.

Without udder lesions there are at least two distinct ways in which *M. tuberculosis* may get into the milk of tuberculous cows; these are as follows:

1. When the organisms are present in the blood. The results obtained by the British Royal Commission[21] show that when *M. tuberculosis* is present in the blood stream it may appear in the milk without causing recognizable udder lesions. The inoculation of cows, either subcutaneously or intravenously, resulted in the presence of the organism in the milk and often it continued to be present for extended periods. In one instance a cow was inoculated subcutaneously with 100 milligrams of a culture of bovine origin and the milk produced tuberculosis in inoculated guinea pigs after 155 days, which was the time of the last examination. The udder appeared normal and showed

---

[18] See ref. 12.

[19] N. Y. (Cornell) Agr. Expt. Sta. Bul. 299. 1911.

[20] See ref. 12.

[21] Final Rpt. Great Britain Royal Com. on Human and Animal Tuberculosis 3. P. 79. 1911.

no lesions or tubercle bacilli on microscopic examination. In 7 of 11 heifers which were inoculated subcutaneously with large doses of *M. tuberculosis*, the organism found its way into the milk sinuses of the undeveloped udders and, in at least 4 cases, was present in such numbers as to suggest multiplication there. While *M. tuberculosis* is not regularly present in the blood of tuberculous cows, it may be under certain conditions, the most common probably being when a lesion ruptures into a blood vessel. The spread of tuberculosis from one part of the body to another rather distant part, as well as the wide distribution of lesions sometimes noted, can best be explained on the basis of the transmission of the organism through the blood stream. In experimental animals the lesions in various parts of the body, following subcutaneous inoculation, also suggest blood transmission although other factors may be involved.

2. When the milk is contaminated after drawing with particles of infected manure. Material coming from the lungs of tuberculous animals is commonly swallowed and thus infects the fecal material. Tuberculous ulcers of the intestinal tract sometimes discharge enormous numbers of organisms and cause the same infection; these lesions appear to be rather uncommon in cows, however. The presence of *M. tuberculosis* in the feces of tuberculous cattle has been studied by a number of investigators and some of the representative results are as follows:

M. Tuberculosis IN FECES OF TUBERCULOUS CATTLE

Investigators	Samples Examined	Samples Positive	Per cent Samples Positive
Schroeder and Cotton *	7	5	71
Reynolds and Beebe †	45	1	2
British Royal Commission ‡	6	5	83
Peters and Emerson §	22	3	14
Briscoe and MacNeal ‖	62	3	5
Moore ¶	36	0	0
Reichel and Deubler **	40	9	22.5

---

* U. S. D. A., B. A. I. Bul. 99. 1907. † Minn. Agr. Expt. Sta. Bul. 103. 1907. ‡ 3rd Interim Rpt. Great Britain Royal Com. on Human and Animal Tuberculosis. P. 14. 1909. § Neb. Agr. Expt. Sta. 22nd An. Rpt. P. 136. 1909. ‖ See ref. 12. ¶ See ref. 19. ** Jr. Med. Res., n.s. 19. P. 5. 1911.

Although the above data show wide variations in the results of different investigations, *M. tuberculosis* is evidently present in the fecal material from certain infected cattle. The infectiousness of feces from tuberculous cattle is also shown by the development of tuberculosis in hogs associated with such material, under both practical and experimental conditions. With the organisms present in manure there is opportunity for the infection of milk under the average conditions of production; the coat of the cow may be contaminated and the fecal material then fall into the milk during the milking process or infection may occur in other evident ways. In this connection the presence of *M. tuberculosis* in the saliva and nasal secretions of tuberculous cows is also of importance. The British Royal Commission[22] noted with several severely infected cows that the uterus was involved and the uterine discharges contained numerous tubercle bacilli.

Under certain conditions *M. tuberculosis* may live for considerable periods in the stable surroundings. Briscoe[23] found that when human and bovine types were exposed to desiccation in a dark, well ventilated place, they were dead within four and eight days respectively, while in direct sunlight they were killed in from one to four minutes. Artificially infected cow manure, exposed to the sunlight in 2-inch layers in a pasture, contained living organisms after two months, although in naturally infected manure the organisms quickly died. These observations indicate that the contamination of milk with manure containing tubercle bacilli may be delayed for a considerable period without the organisms being destroyed.

Growth of M. tuberculosis in milk. Although *M. tuberculosis* develops well on sterile milk held at a favorable temperature, multiplication is not to be expected in milk under practical conditions because the organism has a rather definite growth temperature, especially when it has just come from a lesion, and also because growth generally starts very slowly. Experimental data show, however, that the organism can live for extended periods in milk and this suggests that a long holding period does not make milk safe, as far as this organism is concerned.

[22] See ref. ‡.

[23] Ill. Agr. Expt. Sta. Bul. 161. 1912.

M. tuberculosis in butter. Moore[24] has shown that during the separation of milk containing *M. tuberculosis* many of the organisms are thrown into the slime, while there is a secondary concentration in the cream, and this suggests that the infection of butter is to be expected in certain instances. Briscoe and MacNeal,[25] in 1911, tabulated the results reported in the literature on the presence of *M. tuberculosis* in butter and noted that, of 1,233 samples examined, principally in Europe, 163 or 13.2 per cent were positive. In their own examinations these investigators found that 2 or 33.3 per cent of 6 samples of butter made from cream coming from tuberculous herds contained the organism. Rosenau, Frost and Bryant[26] studied 21 samples of butter secured in Boston; 2 or 9.5 per cent of these contained *M. tuberculosis.* Undoubtedly the percentage of the butter infected with the organism of tuberculosis is less, at present, than formerly due to the extensive use of pasteurization in the butter plants and to the removal of the tuberculous cattle from many herds.

The early studies on the length of time that *M. tuberculosis* will remain alive in butter gave variable results but suggested that the organism persists for considerable periods. More recent data involving modern storage conditions indicate clearly that the organism will live as long as butter is ordinarily stored. Moore[27] reported a trial, made in conjunction with Dawson, in which infected butter still produced the disease in guinea pigs after it had been held 97 days in an ice box. Mohler, Washburn and Rogers[28] found that salted butter stored at either —23.3° C. (—10° F.) or —12.2° C. (10° F.) produced tuberculosis in some of the inoculated guinea pigs after six months storage but not after nine months; one lot of unsalted butter stored at —12.2° C. gave essentially the same result. Tuberculosis was also produced in guinea pigs by feeding the butter but not as regularly as when it was injected. The milk from which three lots of the butter was made was artificially infected but milk from a tuberculous udder was used in inoculating the milk for one lot of salted butter and for the lot of unsalted butter. These investigators call attention to the uneven distribution of *M. tuberculosis* in butter which was shown by samples failing to infect guinea pigs at one examination and then later proving positive. Schroeder and Cotton[29] examined salted butter made from milk coming from a tuberculous udder and found that it contained *M. tuberculosis* up to 160 days when held at 15.6° C. (60° F.). Briscoe [30] reported that *M. tuberculosis* was alive in artificially infected butter stored for 274 days at —10° C. (14° F.), 4° C. (39.2° F.) or approximately 20° C. (68° F.); it was noted that the disease in the inoculated animals was more severe with the butter held at the lowest temperature.

---

[24] U. S. D. A. Yearbook. P. 431. 1895.

[25] See ref. 12.

[26] Jr. Med. Res. n. s. 25. P. 69. 1914.

[27] See ref. 24.

[28] U. S. D. A. B. A. I. 26th An. Rpt. P. 179. 1909.

[29] U S. D. A. B. A. I. Cir. 153. 1910. Also Cir. 127. 1908.

M. tuberculosis in oleomargarine. Briscoe and MacNeal [31] tabulated, from the literature, reports on the examination of 209 samples of oleomargarine for the presence of *M. tuberculosis;* of these, 9 or 4.3 per cent were positive. It seems probable that at least a considerable portion of this infection came from the dairy products used in the manufacturing process, although there are other possibilities, especially infection from human sources. The improved methods of manufacture and handling at present in use with the dairy products employed indicate that they are much less likely than formerly to contain the organism.

Examination of butter for M. tuberculosis. A satisfactory procedure for the examination of butter for *M. tuberculosis* is to put a large sample in a sterile container, add about an equal volume of sterile water or physiological salt solution and, after warming until the butter is thoroughly melted, shake the mixture for 15 or 20 minutes; the material is then cooled and the solidified fat removed. The water or salt solution with the additions acquired from the butter is centrifuged and the sediment used for the inoculation of guinea pigs, either directly or after treatment. While undoubtedly some of the organisms in the butter are held in the fat, many of them appear to be recovered with this procedure and it permits the use of a large sample of the butter.

M. tuberculosis in cheese. The presence of *M. tuberculosis* in milk indicates that it would be carried into the various types of cheeses during the manufacturing processes. Harrison,[32] working at Berne, investigated the survival of this organism in experimental cheese of the Swiss and cheddar types, the cultures being added to the milk; in the Swiss cheese it died between 33 and 40 days while in the cheddar cheese it lived for at least 104 days. *M. tuberculosis* was found in three of five samples of cream cheese secured in Berne, all of which appeared to be only a few days old. This investigator repeated, in Canada, the work on the length of time the organism will survive in cheddar cheese and found that it died out between 60 and 62 days.

---

[30] See ref. 23.

[31] See ref. 12.

[32] U. S. D. A. B. A. I. 19th An. Rpt. P. 217. 1902.

Mohler, Washburn and Doane[33] reported that infected cheddar cheese, secured by adding the organism to the milk, produced generalized tuberculosis in guinea pigs into which it was inoculated when it was 220 days old and that slight lesions were produced after 261 days. Feeding also caused the disease in guinea pigs but not as frequently as inoculations.

Fifty samples of Swiss cheese made of milk from tuberculous cows were examined by Kankoanpaa[34] and seven (14%) contained *M. tuberculosis.* The oldest naturally infected cheese in which this organism was found was 200 days old but the number was low or the virulence weakened. In artificially infected cheese of different types, *M. tuberculosis* lived for varying periods, the maximum being 113 days.

Schroeder and Brett[35] examined cheese, purchased under ordinary market conditions in Washington, for the presence of *M. tuberculosis* with the following results:

M. Tuberculosis IN CHEESE

Type of Cheese	Samples Examined	Samples Positive	Per cent Positive
Cheddar	59	0	0
Neufchatel	32	0	0
Cottage	31	1	3.2
Cream	131	18	13.7
Miscellaneous	3	0	0
Total	256	19	7.4

These investigators pointed out that the infected samples were types that are used in a fresh condition. A second series of tests made on the positive samples, about 60 days after the first tests, gave negative results. Cheese made from milk infected with ground up material from a tuberculous lymph gland of a guinea pig produced tuberculosis in guinea pigs after 1, 8, 15, 23 and 39 days but not after 50, 72, 99 and 114 days.

---

[33] U. S. D. A. B. A. I. 26th An. Rpt. P. 187. 1909.

[34] Centbl. f. Bakt. 1. Abt. Ref. 56. P. 202. 1913.

[35] Jr. Am. Vet. Med. Assn. n. s. 5. P. 674. 1918.

Examination of cheese for M. tuberculosis. In the examination of cheese for *M. tuberculosis* the sample may be ground up with sand and, after filtering through cotton, the filtrate inoculated into guinea pigs, either directly or following treatment. Schroeder and Brett [36] used the following method: Thirty grams of cheese are thoroughly triturated with a freshly made, 2 per cent, aqueous solution of sodium hydrate, using 170 cc. for a soft cheese and double the quantity for a hard cheese. The reagent is added slowly while the cheese is being triturated in a beaker held partly immersed in water at a temperature of 35° to 40° C. (95° to 104° F.). In about 20 minutes this procedure yields a homogenous, turbid fluid which is then centrifuged. If the amount of sediment is small, it is suspended in sterile water and injected subcutaneously into guinea pigs, while if it is large, it should be washed with water before inoculating in order to remove some of the sodium hydrate. These investigators found that a 5 per cent solution of sodium hydrate for 30 minutes did not kill *M. tuberculosis* in cheese so that a 2 per cent solution should never interfere with the results.

M. tuberculosis in ice cream. The occasional presence of *M. tuberculosis* in ice cream can be safely assumed from its general presence in dairy products. The low temperatures used in freezing, hardening and storage would not be expected to destroy the organism completely and, in a trial carried out at the Iowa Agricultural Experiment Station, artificially infected ice cream regularly produced the disease in inoculated guinea pigs; the last examination was made when the ice cream had been held well hardened for 30 days.

Examination of ice cream for M. tuberculosis. Ice cream may be examined for *M. tuberculosis* by melting and inoculating directly or the melted material may be centrifuged and portions of the fat and sediment treated as with milk.

Introduction of tuberculosis into a herd. There are two common methods by which tuberculosis is introduced into a herd, (1) through the purchase of tuberculous animals and (2) through the return, for feeding purposes, of dairy plant by-products, such as skim milk, buttermilk and whey, containing the causative organism.

The danger of introducing a tuberculous animal into a herd through purchase is so great that the tuberculin test is now widely used on animals bought and a careful herdsman will not bring an animal into his herd until its freedom from this disease has been definitely established. Legislation of various types recognizes the possibilities along this line and prevents some of the undesirable practices.

---

[36] See ref. 35.

The return of infected dairy plant by-products has undoubtedly been an important factor in the spread of tuberculosis among farm animals. Moore[37] noted that when milk containing *M. tuberculosis* was separated the skim milk was not freed from this organism. Russell[38] reported some interesting observations showing the importance of infected factory by-products in the spread of tuberculosis among dairy cattle. It was noted that the percentage of animals reacting to tuberculin in the neighborhood of two Wisconsin creameries, the Medina and Oak Park creameries, was higher than the average and also that the young stock raised on the different farms was infected while, in some cases, the mature animals, many of which were brought into the neighborhood, were entirely free.

Of the 43 patrons of the Medina creamery, 36 had their herds tested and 33 contained reactors, with 12 herds showing more than half of the animals diseased. Of the 3 unaffected herds the owners of 2 had moved into the locality within the last year and had brought all of their animals with them. One farmer, who had also come into the section within the year, had 2 reactors in a herd of 28 but these had been bought from other herds. Another farmer, who had only 1 reactor among 48 animals, was a cream patron and the reactor was the only animal in the herd that had been fed on skim milk from the creamery. Of the 784 animals tested, 271 reacted.

Twenty-four of the 36 patrons of the Oak Park creamery had their herds tested and 103 of the 429 animals examined reacted. The disease was found in 14 of the herds but a high percentage of reactors was found in only 4. In 1 herd, 24 out of 26 animals reacted while in another 29 out of 30 reacted. From the histories obtainable it appeared that practically all of the reacting animals had been raised on the farms. It was noted that some time previously a herd tributary to the creamery had been destroyed because of a heavy infection, 27 of 30 animals being slaughtered. This herd may have been an important factor in causing the high incidence in the neighborhood. An inquiry made of the secretary of the creamery developed the fact that, since the creamery was started, the skim milk had been pas-

[37] See ref. 24.

[38] Wis. Agr. Expt. Sta. Bul. 143. 1907.

teurized by exhaust steam at from 60° to 71.1° C. (140° to 160° F.) during the winter months, except during the winter the original heavily infected herd was destroyed, but that during the summer months no attempt at pasteurization had been made.

The percentage of infection in the tested animals in the Medina and Oak Park creameries was 34.6 and 24.0 respectively. Among 1,467 animals tested in 78 herds whose owners patronized 11 neighboring creameries, only 127 or 8.7 per cent reacted.

Financial losses due to tuberculosis in cattle. Although the primary interest in tuberculosis in cattle is due to its relationship to the public health, there are also important economic considerations. The owner of a tuberculous herd may have direct financial losses along two main lines, (1) the death of animals and (2) the decrease in milk production. The loss of animals on a farm after the introduction of tuberculosis is not rapid, as with some diseases, but an occasional death is quite common and herds, which on testing show many reactors, may give a history of a death from time to time over a considerable period. The loss due to decreased production is difficult to determine but it is probable that diseased animals, many of which are emaciated, can not produce as much milk or butter fat as if they were in a healthy condition.

Tuberculosis in cattle is often responsible for the disease in hogs associated with them and thus may cause an indirect loss.

Malta Fever

Malta fever has existed for an indefinite period among the inhabitants of the island of Malta. The causative organism was discovered, in 1887, by Bruce,[39] and in 1893,[40] he named it *Micrococcus melitensis.* Later investigations have shown its relationship to the organism causing contagious abortion in cattle and the two are now distinguished on a varietal basis, the Malta fever organism being called *Brucella melitensis var. melitensis A* and the abortion organism *Brucella melitensis var. abortus.* Because of the prevalence of Malta fever among the British soldiers and sailors stationed on the island, the British government in 1904 appointed a commission to investigate the sources of infection and advise methods of control. As early as 1905 the commission considered that the milk from goats was an important factor in the spread of Malta fever among persons.

---

[39] Practitioner. London. 39. P. 161. 1887.

[40] Ann. Inst. Past. 7. P. 289. 1893.

Subsequent studies indicated that goats were easily infected, that about 50 per cent of them acquired the disease naturally and that the causative organism was eliminated in the milk and urine. Ten per cent of the goats in Malta were found to be eliminating the organism in the milk, and this was highly infectious for monkeys, in which it produced attacks similar to typical Malta fever in man. There is evidence that the elimination of the organism in the urine of goats is a late phenomenon and that this occurs in gushes for short intervals. Goats are evidently infected by consuming contaminated feed and, since the urine of infected goats may contain the organism, there is abundant opportunity for this. In man the most striking symptom of Malta fever is a febrile attack with periods of normal temperatures, the disease commonly running its course in from six weeks to a year; the mortality is estimated at three per cent.[41]

Distribution of Malta fever. Malta fever is especially prevalent in the basin of the Mediterranean but has been reported from many countries and from all of the continents. In 1911, Ferenbaugh and later Gentry and Ferenbaugh[42] established through agglutination tests and blood cultures that Malta fever existed endemically in the goat raising sections of Texas. All the human cases reported by them gave a history of association with the goat industry and some had consumed milk from goats. In an examination of 151 goats these investigators found that about 20 per cent gave a positive agglutination test in a dilution of 1 to 20, while 3 agglutinated in a dilution of 1 to 40. A large number of goats in the section studied were direct descendants of goats imported from districts in which Malta fever is known to exist. Mohler and Eichhorn[43] have substantiated the existence of the disease in Texas and proved its presence in New Mexico by agglutination and complement fixation tests on sera of goats from these states. Furthermore, careful investigations seem to show that the disease has existed for many years in Texas and New Mexico, among people connected with goat raising, under such names as mountain fever and slow typhoid fever. Holt and Reynolds[44] reported that they believed Malta fever is endemic along the entire Mexican border of Texas, New Mexico and Arizona and that many cases in man are overlooked, either by confusing them with some other condition or through inability to diagnose the cases at all.

---

[41] U. S. D. A. B. A. I. 28th An. Rpt. P. 119. 1911.

[42] J. A. M. A. 57. Pp. 730, 889, 1045 and 1127. 1911.

[43] See ref. 41.

[44] Mil. Surg. 56. P. 414. 1925.

Malta fever in goats. Goats infected with Malta fever may harbor the organism for extended periods without showing any evidence of disturbed health. An important symptom among infected goats is the frequency of abortions. The anatomical change most often noted when infected goats are autopsied is an enlargement of the spleen. Mohler and Hart[45] fed cultures of the Malta fever organism to a normal American goat and secured an infection, which was evident from a pronounced increase in the agglutinating power of the blood.

The importance of goats' milk in spreading the disease has been shown in various ways. When the fresh milk supplied the soldiers at Malta was pasteurized the disease among them was reduced 90 per cent. The disease did not occur among the convicts of the civil prison at Malta who were not allowed milk. It was practically eliminated among British soldiers stationed at Gibraltar by prohibiting the consumption of raw goats' milk. Mexican goat herders are only infrequently infected; this may be due to the fact that they always boil the milk before drinking it.

History of a shipment of goats to the United States. The history of a shipment of goats brought to the United States by the Bureau of Animal Industry[46] affords interesting proof of the role played by milk from infected goats in spreading Malta fever to persons. A herd of 61 nannies and 4 billy goats was shipped from Malta, August 19, 1905, on the steamer Joshua Nicholson for the United States via Antwerp. On reaching Antwerp on September 2, the goats remained in quarantine five days and then went on the steamship St. Andrew for New York, arriving September 24. The goats were transferred to the quarantine station at Athenia, New Jersey, where they remained under observation. Bacteriological examination resulted in the recovery of the Malta fever organism, first from the milk of 2 goats and later from that of several more.

On the Joshua Nicholson there were 23 officers and men in addition to the persons in charge of the goats and goat milk was freely consumed. Eleven of the crew left the ship at Antwerp and, with the exception of one who was in the hospital because of a hernia, their movements could not be traced. Eight of the 12 remaining officers and crew fell sick at intervals varying from 18 to 34 days from the embarkation of the goats and, in five of the eight, blood reactions showed that Malta fever was the cause of the illness. Of the four who were not sick, two drank very little milk and the other two appear to have boiled their supply. The steamer St. Andrew carried 30 cattlemen, a crew of 30 and the 4 persons in charge of the goats. Although many of these drank milk there was no evidence of illness. After the arrival of the goats in the United States a number of persons tasted the milk but only two drank any quantity. One of these doubted the idea of Malta fever being conveyed by goats' milk and continued to drink the milk at frequent intervals. He died suddenly, in January, 1906, from pneumonia and the agglutinating power of his blood against the Malta fever organism was never tested. The other person developed a typical case of Malta fever.

---

[45] U. S. D. A. B. A. I. 25th An. Rpt. P. 279. 1908.

[46] U. S. Pub. Health Mar.-Hosp. Serv. Hyg. Lab. Bul. 56. P. 201. 1909.

Example of an epidemic of Malta fever due to goats' milk. Lake[47] reported an epidemic of Malta fever which occurred in Phoenix, Arizona, in 1922, as a result of the consumption of goats' milk. More than 30 cases were positively diagnosed and undoubtedly many others occurred. In all but three of the cases goats' milk from the same dairy was known to have been used and in those three it could not be excluded. The Malta fever organism was recovered from two of the cases. Agglutination tests were made on 115 goats of the herd responsible for the epidemic and 18.3 per cent were positive.

Significance of Malta fever among goats in the United States. Although only a comparatively small amount of goats' milk is consumed in the United States at the present time, an increase in this will undoubtedly occur and, in the development of the goat industry, the possibility of spreading Malta fever to both goats and humans must be given serious consideration.

Contagious Abortion

Contagious abortion in cattle has been known in England and continental Europe for many years. In 1897, Bang[48] ascribed it to a definite organism, which produced the disease on artificial inoculation and which could be recovered from the infected animals. The results of these studies have been widely confirmed and the organism secured by Bang is undoubtedly the common cause of abortion in cattle.

Presence of the abortion organism in milk. Various investigators have observed that guinea pigs inoculated with milk for the purpose of detecting *M. tuberculosis* and autopsied after extended periods, showed lesions which could easily be mistaken for tuberculosis but in which no acid fast organisms could be demonstrated. Schroeder and Cotton[49] noted this condition in guinea pigs in a series of tests relative to the occurrence of *M. tuberculosis* in ordinary milk and found it transmissible from one guinea pig to another, by subcutaneous inoculation of affected tissue. Their interest in the nature of this disease was continued when they found that the milk of a tuberculous cow caused it, even when the milk was secured so as to exclude its contamination from any source but the interior of the udder. The responsible organism was eventually cultivated and its similarity to the organism of contagious abortion led Mohler and Traum[50] to suspect that the two were identical; this suspicion was strengthened by the fact that much of the milk studied by Schroeder and Cotton came from the same sources from which Mohler and Traum had secured material for their work on contagious abortion. A comparison of the organisms showed no detectable difference, either cultural or morphologic, and the use of the Schroeder and Cotton organism as an antigen in applying the complement fixation test to sera from animals infected with abortion, established the identity beyond a doubt. By inoculating the abortion organism Mohler and Traum produced the typical lesions in guinea pigs.

---

[47] Pub. Health Rpts. 37. P. 2895. 1922.

[48] Ztschr. f. Tiermed. 1. P. 241. 1897.

The work of various investigators makes it evident that the organism of contagious abortion is often present in milk. Schroeder and Cotton[51] found, in a series of 77 samples of market milk from 31 dairies, that 8 samples from 6 dairies were infected. In another series of 140 samples from 4 dairies they secured the following results: Dairy A, 35 samples, 11 infected; Dairy B, 33 samples, 7 infected; Dairy C, 34 samples, 2 infected; Dairy D, 38 samples, 2 infected. With a single examination of milk from individual animals, these investigators found that 19 of 140 cows in a large herd in the District of Columbia and 11 of 36 cows at the Bureau of Animal Industry Experiment Station at Bethesda, Maryland, yielded the organism; a considerable percentage of the cows in the latter group were tuberculous. Fleischner and Myers,[52] in 1917, reported that the abortion organism, "is for practical purposes, always present in the certified milk produced in the San Francisco Bay regions." Cooledge,[53] using

[49] U. S. D. A. B. A. I. 28th An. Rpt. P. 139. 1911.

[50] U. S. D. A. B. A. I. 28th An. Rpt. P. 147. 1911.

[51] See ref. 49.

[52] Am. Jr. Dis. Child. 14. P. 157. 1917.

[53] Mich. Agr. Expt. Sta. Tech. Bul. 33. Part 3. 1916.

a method of examining the milk with the agglutination test, secured the following results on the prevalence of udders infected with the abortion organism:

Prevalence of Udders Infected with Abortion Organism

Farm	Cows Tested	Infected Udders	
		Number	Per cent
A	26	0	0
B	40	9	22
C	9	0	0
D	15	5	33
E	13	2	15
F	7	6	86
G	2	2	100
H	6	4	67

Farm A was a certified dairy, while Farms F and G were studied because they were known to have badly infected herds.

Wilson and Nutt [54] found the abortion organism frequently growing in association with *M. tuberculosis* in guinea pigs injected with milk. There was no evidence that this resulted in an activation of the virulence of either organism. About 5.7 per cent of the samples from individual animals and 8.8 per cent of the mixed milks were infected with the abortion organism while 13 per cent of the mixed milks contained *M. tuberculosis.*

Relationship of the Malta fever and abortion organisms. The close relationship of the Malta fever and abortion organisms was noted by Evans,[55] in 1918. Further investigations [56] have shown that the simple agglutination test can not differentiate the two varieties of *Br. melitensis* and it is necessary to resort to the agglutinin absorption test to identify an infection. The condition in man, due to either of the organisms, is commonly called Malta or undulant fever.

Dangers to man from the organism of contagious abortion. The wide range of animal species that are susceptible to the organism of contagious abortion suggests that infections in man are to be expected.

[54] Jr. Path. and Bact. 29. P. 141. 1926.

[55] Jr. Inf. Dis. 22. P. 580. 1918.

[56] U. S. Pub. Health Serv. Hyg. Lab. Bul. 143. Part 1. 1925.

The earlier studies along this line involved the examination of blood for the presence of antibodies; positive results with agglutination and complement fixation tests have been secured with many individuals, both normal and diseased, but the results can not be considered conclusive. Cooledge[57] found it possible to cause antibodies for the abortion organism to appear in the blood serum of adults by feeding a milk naturally infected with the organism and which contained antibodies for it; he considered that these antibodies indicated a passive immunity due to the absorption in the intestine of the antibodies present in the infected milk.

In 1924, Keefer[58] reported a human infection with the abortion organism; the course of the disease was quite like that of Malta fever, as it is observed in regions where this is endemic. There was no evidence that goats' milk or products from goats' milk had been consumed but there had been a heavy use of raw cows' milk. Other reports followed, including cases from various parts of the United States and from a number of foreign countries.[59] Orr and Huddleson[60] reported 16 cases from Michigan in which the diagnosis seemed to be definitely established. From 6 of the patients *Br. melitensis var. abortus* was isolated from either the blood or urine, while the remaining 10 gave a typical clinical syndrome and an agglutination with the organism in dilutions ranging from 1:640 to 1:10,000. None of the patients had been in contact with goats but all had been consuming raw cows' milk in one way or another at the time of and previous to the onset of the symptoms. Hardy[61] reported agglutination tests for Malta fever on blood specimens sent to a public health laboratory. Of 783 tests, 68 were positive; because of duplications the 68 tests involved 38 cases, most of which were confirmed clinically.

It appears that as the possibility of human infection with *Br. melitensis var. abortus* becomes better known, more and more cases are being recognized. In the past, errors in diagnosis have probably tended to conceal such infections. The surprising thing is that, with the great prevalence of contagious abortion among cattle and the elimination of organisms through the milk by infected animals, more human cases are not encountered. The explanation of this may be

[57] Mich. Agr. Expt. Sta. Tech. Bul. 33. Part 2. 1916.

[58] Johns Hopkins Hosp. Bul. 35. P. 6. 1924.

[59] U. S. Pub. Health Serv. Hyg. Lab. Bul. 143. Part 4. 1925.

[60] Am. Jr. Pub. Health. 17. P. 1242. 1927.

[61] Pub. Health Rpts. 43. P. 503. 1928.

a considerable resistance on the part of most persons so that a definite illness occurs only with those having a lowered resistance or when the organisms are ingested in unusually large numbers.

Methods of examining milk for the abortion organism. The inoculation of guinea pigs is a satisfactory method of examining milk for the presence of the abortion organism. It has the disadvantage of usually requiring from six to ten weeks for the lesions to be pronounced in the experimental animals, although occasionally development is more rapid. The inoculations can be made intraperitoneally, using milk direct or a mixture of sediment and cream secured by centrifuging. The lesions are of various types but usually a more or less characteristic enlargement of the liver and spleen is noted. The organism should be isolated by culturing the liver and spleen on some medium, such as beef infusion agar or beef infusion agar to which blood has been added, and its identity then established by cultural and serologic tests. The cultures for isolation should be held in a container in which about 10 per cent of the air has been replaced by carbon dioxide, although cultures are sometimes secured without any such replacement.

Cooledge[62] investigated the antibody content of milk, using the agglutination and complement fixation tests, as a means of locating udders infected with the abortion organism. The milk serum was secured with rennin. The agglutination test gave a higher percentage of positive reactions and appeared to be the more reliable. Whenever the abortion organism was found in the milk by animal inoculation the agglutinins for this organism were also found, but the organism was not present in every case in which the agglutinins were demonstrated. The agglutinins were present in the milk of cows that had never aborted as well as in the milk of cows that had. From the results obtained it appeared that the antibodies may be present in one or more quarters, may spread from quarter to quarter and may gradually die out. The variations in the antibody content of the milk from different quarters suggest that the antibodies do not come from the blood but are formed in the udder. Antibody and body cell contents in the strippings as high as those in the fore milk seemed to indicate active infection and the presence of the abortion organism in the milk in sufficient numbers to produce typical lesions and blood reactions when five cubic centimeters were inoculated into the abdominal cavities of guinea pigs; lower antibody and body cell contents in the strippings than in the fore milk seemed to indicate

[62] Mich. Agr. Expt. Sta. Tech. Bul. 33. Part 1. 1916.

that infection was slight and that the abortion organism was not present in sufficient numbers to cause infection with the five cubic centimeters used for inoculation. Cooledge reported that the agglutination test is of value in studying the presence of the abortion organism in milk when it is desired to study a large number of samples.

Danger of spreading the abortion organism through factory by-products. The presence of *Br. melitensis var. abortus* in milk is of significance from the standpoint of its spread through dairy plant by-products returned to the farms for feeding purposes. It should be noted, however, that this organism is primarily a cattle type and that other animals may show considerable resistance to it. Huddleson and Hasley[63] have secured data which indicate the improbability of *Br. melitensis var. abortus* persisting in the tissues of calves from a pre-natal infection or from the ingestion of milk containing the organism. This suggests that cattle may be more susceptible when the breeding age is approached.

Foot and Mouth Disease

Foot and mouth disease is a highly contagious disease that affects various domestic and wild animals, especially cattle, and is sometimes transmitted to man. The virus is present in the fluid of the vesicles and may gain entrance to the saliva, feces, urine and milk from this source. Lesions sometimes occur on the udder; in certain instances the milk ducts and even the secreting portions of the udder are involved and the milk is then highly infectious. There appears to be some evidence that the milk does not contain the virus when the udder is free from lesions. Early in the disease the virus is present in the circulating blood.

Foot and mouth disease causes a reduction in the milk flow in cows and the milk produced may be very abnormal in appearance and taste. Wing[64] reported that the daily milk production of an infected herd dropped from 4,000 to 1,200 pounds in a period of six days.

The disease in man may be contracted by contact with diseased animals or through the ingestion of infected milk, cream, butter, cheese, etc. In European countries, where foot and mouth disease has been common, many cases in man have been reported, sometimes in epidemic form. Children appear to be especially susceptible. The disease in man usually takes a mild form, although occasionally the symptoms may be severe and some cases end fatally. The period of incubation in man varies from two to ten days or more.

---

[63] Mich. Agr. Expt. Sta. Tech. Bul. 66. 1924.

[64] Int. Assn. Dairy and Milk Insp. 13th An. Rpt. P. 166. 1924.

There have been a number of outbreaks of foot and mouth disease in the United States, primarily among cattle, but these have been stamped out by quarantine and the destruction of the infected herds. Occasional cases of foot and mouth disease in man have also occurred in the United States. Clough [65] reported one from Baltimore; experimental proof of the nature of the disease could not be secured through animal inoculation but the clinical picture was characteristic. The infection may have come from milk or cream since the disease was present in cattle in the neighborhood at the time. Sutton and O'Donnell [66] reported a case from Kansas in which the diagnosis was also based primarily on clinical evidence. In an outbreak in California, Wing [67] failed to find a case of foot and mouth disease in man under conditions which seemed to be favorable for its spread since, before the disease was recognized, raw milk from infected farms was sold.

Milk or milk derivatives may be a means of spreading foot and mouth disease from one farm to another. Wing [68] has recorded an instance in which milk from an infected herd was separated and the raw skim milk shipped to another farm where it caused foot and mouth disease in the hogs to which it was fed; the disease then spread from the hogs to the cattle. There was no connection other than the milk between the two farms which were 57 miles apart.

JOHNE'S DISEASE

The spread of Johne's disease from one farm to another through dairy plant by-products is suggested by the general nature of the condition. Portions of the intestinal wall are rather characteristically thickened and contain large numbers of the responsible organisms so that the fecal material may be readily infected. Hastings, Beach and Mansfield [69] have pointed out that the desquamation of the epithelium is probably rapid in the areas in which the organism is growing and this suggests that the shedding of the organism would be a steady process rather than an intermittent one. The contamination of milk with infected fecal material would add the organism to the milk and the transfer of this to other farms, either as such or as skim milk or buttermilk, could spread the disease in quite the same way that tuberculosis is spread. The use of infected milk for feeding calves on the farm where it is produced may also be a factor in extending the disease.

---

[65] Johns Hopkins Hosp. Bul. 26. P. 351. 1915.

[66] J. A. M. A. 66. P. 947. 1916.

[67] See ref. 64.

[68] See ref. 64.

[69] Wis. Agr. Expt. Sta. Res. Bul. 81. 1927.

The increasing importance of Johne's disease and the possibility of tremendous economic losses from it, suggest that every precaution should be taken to prevent its spread.

ANTHRAX

Bacillus anthracis may be present in the milk of cows that have anthrax. Moore[70] stated that Heusinger reported anthrax in man produced by drinking milk from a cow affected with the disease. M'Fadyean[71] examined three samples of milk drawn post-mortem from cows that had died of anthrax and, in each, anthrax bacilli were so numerous they could readily be detected by microscopic examination. In an outbreak studied by Moore[72] the organisms were not found in the milk until just before or soon after the death of the cows. About 14 animals died and, for three weeks after the first case appeared and before the diagnosis was made, the milk from the herd was retailed without injury to the consumers. This investigator has suggested that the elimination of organisms with body discharges may afford an additional opportunity for the contamination of milk.

It seems probable that the milk of cows having anthrax is not infected with *B. anthracis* until active multiplication of the organism in the blood stream begins. This usually occurs only shortly before death and is accompanied by a temperature increase and other evidences of an abnormal condition, among which there is ordinarily a suppression of the milk secretion and a change in the character. Accordingly, there is little danger from the milk of a cow having anthrax until the disease has progressed to a point where an abnormal condition is very evident. The general measures necessary to control the spread of anthrax from an infected herd make it advisable to prevent the shipment of the milk from the normal cows.

[70] See ref. 24.

[71] Jr. Comp. Path. and Ther. 22. P. 148. 1909.

[72] N. Y. State Jr. Med. 19. P. 138. 1919.

ACTINOMYCOSIS

The possibility of man acquiring actinomycosis through the consumption of milk from infected cows appears to be very slight. If the udder is involved the danger from *Actinomyces bovis,* as well as from secondary invaders that may be present in the lesions, is presumably greater than when the lesions are in other parts of the body. Cases of actinomycosis in man sometimes appear to be the result of infection through injuries to the mucous membranes of the intestinal tract but the organism probably must be carried into the tissues.

Actinomycosis of the udder seems to be rather uncommon in the United States. Kendall[73] has, however, reported a large number of cases among the cows in Victoria which may be the result of an unusual prevalence of the causative organism on the pastures; the hind quarters were found to be involved more frequently than the fore quarters.

RABIES

Although cows occasionally develop rabies there is little danger of the spread of this disease through milk. Peters[74] reported that on several occasions the inoculation of rabbits and guinea pigs with milk from a rabid cow had given negative results. Nicolas[75] found that the milk and mammary tissue of herbivorous animals suffering from rabies was never virulent.

UDDER INFLAMMATIONS

The relationship of organisms to udder inflammations was early recognized as a result of the development of a mastitis when materials containing organisms were injected into the udder through the teat canal and by the appearance of an inflammation when a normal udder was injected with material from one that was diseased. Variations occur in the type of udder inflammation, partly through differences in the causative organisms but also because of differences in the resistance of the tissues.

Extent of the inflammation. Frequently, only one quarter of the udder is abnormal but in other cases two or even more quarters are involved. The condition may be so slight that it is recognized only on very careful examination or so pronounced that the tissues are

[73] Vet. Jr. 70. P. 132. 1914.

[74] Chapin, C. V. The Sources and Modes of Infection. P. 366. 1912.

[75] Jr. Med. Vet. et Zootech. 56. P. 721. 1905.

sensitive, feverish and greatly indurated. With only a slight tissue involvement the milk may be normal in amount and appearance while, with the severe condition, it may be greatly reduced in amount and have an abnormal appearance. Sometimes difficulty in straining is the first indication of an abnormality. Milk from an infected udder often contains a large number of leucocytes and, when the milk is very abnormal in appearance, the numbers are commonly excessive. Many infected udders quickly return to normal and proper care is an aid in this. Occasionally, one or more quarters may be so severely involved that milk secretion ceases and that portion of the udder is lost for the time being or possibly for succeeding lactation periods also.

Seasonal variations. Although udder inflammations occur throughout the year, in many dairy sections of the United States they appear to be more common during the spring and fall. During these seasons the cows are sometimes left out over night when the temperatures are quite low and the chilling of the udder tissue may so lower the resistance that organisms can establish themselves.

Variations in susceptibility of different cows. Cows vary a great deal in their susceptibility to udder inflammations, some animals having little or no difficulty of this sort while others frequently develop abnormal udder conditions. Occasionally, an animal is encountered in which one or more quarters shift back and forth from a normal to an abnormal condition throughout an entire lactation period. Individual susceptibility appears to be a big factor in accounting for frequent udder inflammations since these may occur in animals that receive excellent care.

Epidemics of udder inflammation. Usually the percentage of cows in a herd that have udder infections is small, but in some instances the condition appears in epidemic form and involves a large percentage or all of the cows. Such outbreaks appear to be due to organisms having an unusually high virulence; under the average stable conditions they can readily be carried from one cow to another by the milker or in some other way.

Types of organisms causing udder infections. Among the organisms responsible for udder infections streptococci occur most frequently while staphylococci are second in importance. Savage[76] investigated 36 cases of mastitis and found 68 per cent due to streptococci, 16 per cent to staphylococci, 3 per cent to *E. coli,* 3 per cent to *M. tuberculosis* while with 10 per cent the results were doubtful. In 43 cases studied by Stader,[77] 74.4 per cent were due to streptococci, 16.4 per cent to staphylococci and 9.3 per cent to the Escherichia-Aerobacter group.

---

[76] Savage, William G. Milk and the Public Health. P. 107. 1912.

The Streptococcus most frequently met with in cases of mastitis is *Streptococcus mastitidis* and this appears to be the typical organism of infected udders. It is very similar to *Streptococcus pyogenes* but, according to Ayers and Mudge,[78] can be distinguished by the lower *p*H produced in dextrose yeast broth and by the hydrolysis of sodium hippurate into benzoic acid and glycocoll; these investigators reported two types of *S. mastitidis* that vary in the type of reaction produced on blood agar plates. It appears from the work of Ayers and Mudge that *S. mastitidis* may be found in normal udders, although in smaller numbers than in cases of mastitis, and this indicates that, when the resistance of the tissues is lowered, definite infections develop as a result of the activity of organisms regularly present. Such a relationship is also suggested by the fact that, when only one quarter shows an abnormal condition, one or more of the other quarters may contain the same organism as the diseased quarter.

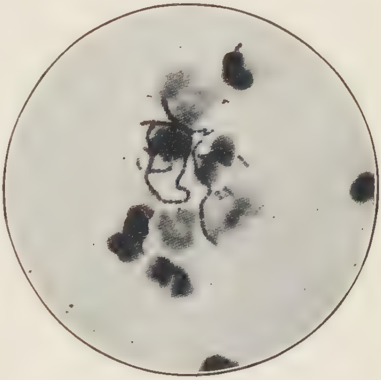


Fig. 22.—Streptococci and leucocytes from an abnormal udder.

S. mastitidis is a typical bovine type that appears to be nonpathogenic for man. Savage [79] unsuccessfully attempted to infect his own throat with massive doses and undoubtedly a great deal of milk containing this organism has been consumed without harmful results. Milk from infected udders is often used for the feeding of farm animals.

The streptococci other than *S. mastitidis* that are found in cases of mastitis show considerable variation. In some instances an organism has been secured that appeared to be the same as the one responsible for an epidemic of septic sore throat. The organisms other than streptococci from infected udders may or may not be harmful when consumed by man. In general it can be said that the organisms

[77] N. A. Veterinarian. 3. P. 133. 1922.

[78] Jr. Inf. Dis. 31. P. 40. 1922.

[79] See ref. 76.

responsible for most of the udder infections are non-pathogenic for man but that there are occasional pathogenic types and these create a public health problem in connection with udder infections.

Milk Sickness or Trembles

Milk sickness or trembles is primarily a disease of cattle and other farm animals and occurs in man through the consumption of milk, milk derivatives or the flesh of affected animals. It has been known for many years, at least since shortly after the middle of the eighteenth century, and was encountered by the early settlers in many sections of the United States. Certain areas were regarded as milk sick areas and were carefully avoided or, in some instances, fenced. As the country became settled the disease gradually decreased until now it has disappeared in areas where it was once common. Wolf, Curtis and Kaupp [80] stated, in 1918, that the disease probably occurs in the mountainous portions of all the counties of Western North Carolina and in the immediately adjacent sections of states having similar conditions. In 1926, Marsh, Roe and Clawson [81] reported that a disease of cattle, commonly known as alkali poisoning or milk sickness, has long been known in the Pecos Valley of New Mexico and Texas and that a disease in man has generally been identified with it.

Theories as to the cause of milk sickness. As has been pointed out by Wolf, Curtis and Kaupp,[82] the theories as to the cause of milk sickness have been along three main lines, (1) mineral poisons, (2) micro-organisms and (3) poisonous plants.

1. *Mineral poison theory.* A number of observers and investigators have considered that milk sickness was due to mineral poisons secured in one way or another and the minerals that have been held responsible include arsenic, copper, mercury, cobalt, lead and aluminum. The evidence at present available makes this theory untenable.
2. *Micro-organism theory.* For a long time micro-organisms were considered to be a possible cause of milk sickness. Jordan and Harris [83] studied an organism, which they named *Bacillus lactimorbi,* in its relationship to the disease and further studies were carried out by Luckhardt,[84] but in neither investigation could

---

[80] N. C. Agr. Expt. Sta. Tech. Bul. 15. 1918.

[81] U. S. D. A. Bul. 1391. 1926.

[82] See ref. 80.

[83] Jr. Inf. Dis. 6. P. 401. 1909.

[84] Jr. Inf. Dis. 6. P. 492. 1909.

the organism be definitely established as the cause. Jordan and Harris isolated *B. lactimorbi* from the heart blood and certain internal organs of animals examined a few hours after death from milk sickness.

3. *Poisonous plant theory.* Poisonous plants were considered to be the cause of milk sickness by some of the early observers of the disease. This theory was supported by a number of facts, such as the time of year the disease is most common and its disappearance as the land is cultivated. Although a large number of plants have been accused, white snakeroot or richweed has been blamed most often and the data collected in recent years prove quite conclusively its etiologic role in at least certain cases of the disease.

Plants important in causing milk sickness. In 1917 results of various investigations were reported which definitely established the importance of white snakeroot in milk sickness. Curtis and Wolf[85] developed 15 cases in sheep by feeding this plant, 14 of which resulted fatally; death occurred in from 5 to 27 days following the beginning of the feeding. Marsh and Clawson[86] found in their studies that white snakeroot was toxic for both cattle and sheep. Moseley[87] noted that an ether extract of the leaves of white snakeroot, when consumed by rabbits, affected them similarly to the leaves themselves. In 1918, Wolf, Curtis and Kaupp[88] reported an extended series of experiments in which they were able to produce milk sickness in various animals by feeding white snakeroot. The investigations were carried out at West Raleigh, North Carolina, where no case of the disease had ever appeared prior to the trials, and the white snakeroot used was shipped 400 miles. During the studies, 31 fatal cases developed among 44 ewes and lambs used in various phases of the work, 1 of the 2 hogs employed development typical trembles and 15 of 29 guinea pigs used died from eating white snakeroot or its products. Two lambs contracted the disease by suckling their mothers, proving that the disease may be transmitted through milk. It was not possible to determine with certainty whether or not death resulted from the feeding of butter in the case of the mice employed. Animals appeared to differ greatly in their susceptibility to poisoning with white snakeroot. Sackett[89] reported feeding experiments with white snakeroot and with various extracts of it, which indicated that guinea pigs are more resistant to poisoning than rabbits. The viscera and flesh of rabbits which had died from the poisoning were without harmful effect when fed to a cat.

---

[85] Jr. Agr. Res. 9. P. 397. 1917.

[86] Jr. Agr. Res. 11. P. 699. 1917.

[87] Med. Rec. 92. P. 428. 1917.

[88] See ref. 80.

The results of Marsh, Roe and Clawson,[90] who studied the milk sickness encountered in the Pecos Valley, showed that typical symptoms could be produced in cattle, horses and sheep by feeding sufficient quantities of rayless goldenrod. It was found that the toxic substance could be excreted in the milk and that calves and lambs may be poisoned in this way; these findings suggest that cases in man may occur from the consumption of milk or butter from cows feeding on the rayless goldenrod.

The toxic constituent of white snakeroot. The investigations of Couch[91] indicate that tremetol, $C_{16}H_{22}O_3$, is the constituent of white snakeroot that is responsible for milk sickness. This compound has the properties of an unsaturated alcohol and is soluble in fat and fat solvents; it may be secreted in milk and, because of its solubility, would be carried into butter. A resin acid and a volatile oil are also present in white snakeroot but are incapable of producing milk sickness. When white snakeroot is dried the tremetol content rapidly decreases and the completely dried plant does not produce milk sickness. The toxic constituent of the rayless goldenrod is not destroyed by drying.

GROUP 2. DISEASES IN WHICH THE INFECTIOUS MATERIAL COMES FROM MAN DIRECTLY OR INDIRECTLY

In the United States the most important of the milk-borne diseases belonging to the group with which the infectious material comes from man, either directly or indirectly, are typhoid fever, septic sore throat, scarlet fever and diphtheria. Although the organisms responsible for septic sore throat sometimes come from the udders of the producing animals they are primarily human types and the udders may be infected from human sources. When milk-borne, the diseases in this group often occur in epidemic form in the same general manner as when they are spread in other ways. However, milk-borne epidemics have certain characteristics and a knowledge of these is

[89] Jr. Inf. Dis. 24. P. 231. 1919.

[90] See ref. 81.

[91] Jr. Agr. Res. 35. P. 547. 1927.

often helpful in deciding whether or not an epidemic under investigation is due to milk.

Important characteristics of milk-borne epidemics. The more definite characteristics of milk-borne epidemics and the ones of most help in recognizing infected milk supplies are the following:

1. The cases are largely confined to the users of milk from one source. If an epidemic is due to milk from a certain source it would be expected that the cases would be largely confined to persons consuming milk from the dealer handling it, while epidemics from other sources, such as water, would not be expected to follow a given milk supply. Accordingly, when a considerable percentage of the cases in an epidemic are along the route or routes of a dealer, a milk-borne outbreak is at once suggested. It must be recognized, however, that if a dealer furnishes a large part of the milk sold in a community his supply can be expected to have a high percentage of the cases in an epidemic, even when milk is not involved; for example, if a dealer sells 95 per cent of the milk, he may have about 95 per cent of the cases in an epidemic due to city water or some such cause. In general, with the usual complexity in the milk distribution, any considerable number of cases among the consumers of a given milk supply and not among the consumers of other supplies is suspicious.

    There are various factors which tend to limit infections among persons consuming milk from an incriminated supply, some of which are as follows:

    (*a*) Not all milk contains the responsible organism. There is no reason to believe that every portion of a supply causing an epidemic contains the responsible organism and undoubtedly some households escape in many epidemics because the milk coming to them happens to be free from the pathogens.

    (*b*) Some milk is heated before it is consumed. In certain homes milk is commonly heated, often to boiling, before it is consumed and with sufficient heating the pathogens present are destroyed. When the milk is used entirely for cooking or baking there is ordinarily no danger from it.

    (*c*) Resistance of certain consumers. Persons vary in their resistance to different diseases and it is probable that in most milk-borne epidemics certain individuals escape through their immunity. The increasing use of the methods of immunizing against various diseases is of importance in this connection.

The percentage of the persons using an infected supply that contract the disease varies greatly, due to the above factors. In some instances it is very low and in others it may be nearly 100 per cent. Cases of undoubted authenticity have occurred through the drinking of only one glass of milk.

When two dealers get their supplies, in whole or in part, from the same producing farm, one may have cases among his consumers and the other not, due to the infection occurring after the milk leaves the farm; even with a farm infection the supply of one dealer may be made safe by efficient pasteurization while the other is not.

2. Epidemics are often explosive in nature. With milk-borne epidemics of any considerable magnitude a number of cases are often reported at about the same time. This is due to the fact that the infection of the milk is commonly extensive enough so that a number of persons acquire the pathogens at about the same time and, with a fairly definite incubation period, this results in a number of cases appearing simultaneously. In some instances a given supply may be infected over a considerable period and cases then continue to appear, while in others the milk may be infected only once or twice and the cases all be reported in a comparatively short time. Two or even more cases may develop in a family on the same day.

   In addition to epidemics due to milk those spread through general water or food supplies may also be explosive in nature. Accordingly, the simultaneous appearance of a number of cases of one of the milk-borne diseases suggests a general source of infection, with milk one of the possibilities.

3. Most cases are in homes consuming considerable milk. If an epidemic is milk-borne it would be expected that, with a fairly uniform distribution of the pathogens throughout the milk supply, the homes consuming large quantities of milk would show more cases than those using little milk, provided the milk was not treated by a method which would destroy the infectious material. In the past the families with the smaller incomes have often materially limited their milk consumption so that, in some milk-borne epidemics, the cases have been more numerous in families having moderate or large incomes. With the present agitation for an increased use of dairy products, milk consumption may be rather heavy even in families having comparatively small incomes.

4. Most cases are among heavy milk drinkers. The heavy milk consumers in a family have a greater chance of becoming infected during a milk-borne epidemic than the light milk consumers and proportionately more cases occur among them. Children, in general, consume considerable milk and might be expected to show a high case incidence but the milk they consume is often heated so that they may show as low or even a lower rate of infection than adults.

Minor characteristics of milk-borne epidemics. In addition to the characteristics listed above a number of others that are more or less unusual have been noted in certain milk-borne epidemics. The incubation period has sometimes been reported as rather short for epidemics in which the date of the infection of the milk was established. The suggested causes for this are heavy infections, due to growth of the organisms in the milk, and the rapid absorption of the organisms. Certain epidemics have shown a low mortality but with others the mortality has been normal or even high. Milk-borne epidemics have been more common in medium sized and smaller towns than in cities; the reason for this is that efficient pasteurization has been infrequent in the smaller communities and, accordingly, less of the milk has been subjected to a process which makes it safe. Some of the milk-borne epidemics that have occurred in cities have been very extensive, however, due to the wide distribution of the milk.

Methods of tracing epidemics to milk supplies. The methods of tracing epidemics to milk supplies are epidemiological rather than bacteriological. The isolation of the pathogens from a suspected supply is not attempted for the following reasons:

1. Satisfactory methods are not available and the failure to find the organism involved in the epidemic would be no assurance that it was not there.
2. The time required to examine milk for any of the pathogens is considerable and a decision as to the source of the infectious material must be made quickly so that measures can be taken to stop the epidemic.
3. The organism responsible for a milk-borne epidemic is not continuously present in the supply and, since the cases must have been infected some time before they began to appear because of the incubation period, it is quite possible that a supply may have caused the trouble and still not contain the responsible organism at the time an examination would be attempted.

The usual procedure is to secure all of the available data on each case, either direct from the individual or from someone else who is thoroughly informed, and then analyze them in an attempt to locate some possible source of the organism that is common to all or the great majority of the cases. If practically all the cases is an epidemic have used a certain milk supply and have had nothing else in common, the milk is at once under serious suspicion. The data secured may point to some source such as water, foods of various sorts, contact with certain diseased individuals, etc., in quite the same way that milk is indicated. A spot map illustrating the distribution of the cases along the different milk routes is very valuable when milk is considered to be a possible source of the infection, since this shows quickly and in a striking manner any grouping of cases along a particular route. The point to be especially considered is whether or not a route shows an excess of cases over the number to be expected. If a given route has 80 per cent of the cases but at the same time is delivering about 80 per cent of the milk in the locality, it has the percentage that would be expected if the source was not milk and it can not be said to have an excess of cases. However, 80 per cent of the cases occurring on a route supplying only a small percentage of the milk is at once suspicious if nothing else common to the cases is detected.

Considerable care is necessary in securing the data on the cases since some little incident that is not readily recalled may be important in tracing the epidemic. A single glass of milk from a supply other than the usual one may be the only connection between a given case and the milk causing the epidemic and, if this is recorded in the data, it furnishes valuable confirmatory evidence when the source of the epidemic is not clear.

The tracing of an epidemic is essentially an elimination process in which one possibility after another is excluded until the evidence finally points to one remaining source. The possibilities to be considered in an investigation depend on the particular disease involved; for example, the common sources of diphtheria epidemics are quite different than those of typhoid epidemics. A water supply so carefully controlled that it is known to be safe ordinarily makes the detection of milk-borne typhoid much easier than when an uncertain water supply must be taken into account. In reporting to health departments cases of diseases that may be spread through milk, there is an advantage in including the sources of the milk supplies since a tabulation of such data is likely to show at once a distribution of cases indicating a milk-borne epidemic and may lead to a quick recognition of an infected supply.

If the findings incriminate a milk supply, valuable confirmatory evidence may be secured through the location of the source of the organisms, for example, a carrier, a definite case, an infected water supply on the farm, etc. When a suspected supply is stopped or pasteurized and the outbreak subsides, the exact time depending on the incubation period of the disease in question, further proof is provided.

Typhoid Fever

A large number of epidemics of typhoid fever in various countries have been traced to milk and its derivatives while undoubtedly many others have been included with those epidemics that have gone unsolved. Armstrong and Parran[92] have compiled data showing that up to January 1, 1927, the literature contains records of 613 typhoid fever epidemics due to dairy products in the United States alone; these investigators summarized 479 epidemics while 134 were previously tabulated. On the basis of very incomplete data the 479 epidemics involved 14,968 cases and 219 deaths. Milk was considered to be the medium of infection in 444, ice cream in 32, butter in 2 and cheese in 1. The milk was reported as raw in 133 outbreaks and as pasteurized in 21; many of the epidemics in which the type of milk was not given involved small supplies so that it is probable they were largely due to raw milk. If the milk and ice cream outbreaks are combined, there were 29 reported as due to pasteurized supplies. In 12 of these the evidence indicated infection subsequent to the heating, in 3 there may have been a substitution of raw milk and in 3 there was evidence that the heating was not to the specified exposure; in a number of epidemics the pasteurizing equipment was unsatisfactory.

In addition to the epidemics of milk-borne typhoid, isolated cases involving this type of infection undoubtedly occur. These are very difficult to trace and, accordingly, go unrecognized for the most part.

Examples of milk-borne typhoid. Ex. 1. A typhoid epidemic[93] occurred at Ames, Iowa, in 1900 and involved principally the students attending Iowa State College. At that time many of the students used a common dining hall. Shortly before the epidemic began the usual milk supply from two farms became insufficient and additional milk was secured from a third farm. The use of the new supply was soon followed by the appearance of cases of typhoid among the persons eating in the dining hall. Those who ate at the tables furnished with milk from the new supply showed a high case incidence and, of the 16 football men who ate at one table and were encouraged to drink milk, 13 became ill. There were no cases among persons who did not drink raw milk and everyone that became ill had consumed milk freely. In all there were 65 cases with 2 deaths. Investigation showed that on the farm supplying the infected milk there had been a case of typhoid shortly before and that, at the time of the epidemic, the well from which water was taken to wash the dairy utensils was badly polluted. The milk was delivered in unscalded cans about 8 or 9 A.M. and was kept all day and used for the evening meal so that there was abundant opportunity for the growth of organisms.

---

[92] Pub. Health Rpts. Sup. 62. 1927.

[93] Ia. State Bd. Health. 11th Bien. Rpt. P. 96. 1901.

Ex. 2. Bolduan and Krumwiede[94] reported a milk-borne typhoid outbreak in which there was a normal or contact carrier and a chronic carrier on a farm; the former was at first assumed to be the source of the infection while the latter was subsequently found to be responsible.

Eleven cases of typhoid were directly traced to milk from Dairy B and two additional cases probably came from the same source. The dates of onset of the cases extended from October 15 to November 16. The dairy distributed about 880 quarts of milk per day and obtained its supply from six farms; about 160 quarts from one farm were distributed as ''Baby's Milk.'' The milk received at the dairy was bottled and placed in ice boxes in the order of the numbers given the farms, except for the ''Baby's Milk'' which was usually bottled on its arrival in the freshly sterilized bottling equipment. Driver P took his supply first and the others then took their supplies in a rather definite order; all took some ''Baby's Milk'' but P took about one-half of it.

The cases of typhoid were all on P's route due to his supply coming entirely from Farm 1, which proved to be the source of the infection. The contamination of the bottling tank was evidently not serious as the milk subsequently bottled was not infectious.

The farms were inspected and blood and feces were secured from all individuals on the farms and in the dairy. The specimens from two of the men working on Farm 1, S.M. and L.M., were the only ones positive. S.M., who denied having had typhoid, gave a suspicious Widal while two fecal specimens were negative. L.M. also gave no history of typhoid; a Widal was negative while one fecal specimen was positive and another negative. The evidence suggested L.M. as the cause of the typhoid cases but later evidence showed this conclusion to be erroneous.

---

[94] Pub. Health Rpts. 32. Part 2. P. 1755. 1917.

L.M. left the farm but further fecal examinations over a period of six months gave only negative results. S.M. left the farm at about the same time but, in February, returned and then, in March, two cases of typhoid developed, both of whom received milk that did not go to Dairy B but went directly from Farm 1. The examination of a fecal specimen from S.M. gave positive results. Four other typhoid cases were later reported, all attributable to milk from Farm 1. It thus appears that the absence of S.M. and not L.M. was the reason why no cases occurred between the two outbreaks.

The findings indicate that S.M. was a chronic carrier but at the time of the first investigation was eliminating no typhoid organisms or such small numbers they could not be detected by the methods employed. Later his condition was recognized because more organisms were being eliminated. L.M. was a transient normal carrier infected through the milk or by contact with S.M.; the presence of such a carrier may lead to erroneous conclusions in attempts to locate the cause of typhoid epidemics.

Ex. 3. Geiger and Kelley[95] have reported an instance in which milk from a dairy farm was responsible for typhoid in Richmond, California, where it was sold in a raw condition, but caused no typhoid when sold after pasteurization in Berkeley, California. The dairy involved employed 20 men and milked approximately 300 cows. About 90 gallons of milk per day were delivered to Substation 1 in Richmond, from which it was sold separately from milk obtained elsewhere, while about 600 gallons per day were furnished to Substation 2 for delivery in Berkeley. About 500 persons received raw milk from the dairy through Substation 1 and about 6,000 received pasteurized milk through Substation 2. Twelve cases of typhoid with no deaths occurred in Richmond, while no cases traceable to the milk supply in question were found in Berkeley. At the time of the investigation no one ill with typhoid was found at the dairy, but the head milker had been taken ill shortly before and was in a hospital with typhoid as shown by the Widal test. It is of interest to note the small number of cases (12) in comparison to the number of persons (500) consuming the infected milk.

[95] J. A. M. A. 66. P. 263. 1916.

Ex. 4. Evidence[96] was secured in New Jersey indicating that two milk-borne typhoid epidemics occurring about a year apart were due to the same carrier. In the fall of 1921 an outbreak of 72 cases occurred. An investigation showed that one of the dairy workers was a carrier and, since no other probable source of the organisms was found, he was believed to be responsible for the epidemic; typhoid organisms were isolated from two stool specimens. In the fall of 1922, an epidemic occurred in Newark, 30 cases being reported between September 9 and October 23. Twenty-four cases developed in families supplied with raw milk from Dairy X and a study of these failed to reveal any other probable source of infection. The man who had been identified as the carrier in the epidemic the previous fall was located among the workers at the dairy. Under an assumed name he had been employed as a hand and was later assigned as a milker; the typhoid organism was again isolated from stool specimens. The milk was ordered pasteurized and the outbreak stopped.

Ex. 5. Hill[97] investigated a small typhoid epidemic at North Branch, Minnesota, that led to some interesting evidence with reference to the dangers from a carrier. Of the ten cases, seven appeared to be primary and three secondary; assuming the usual incubation period, four of the primary cases were infected about the middle of January and three at various times in February. Approximately 100 of the 150 families in North Branch obtained milk from cows kept by themselves or their immediate neighbors, while two dairies each supplied about 25 families. At the time of the investigation the families having cases had no common milk supply, but on the probable dates of infection each of these families had been a user of the milk from one dairy, designated Dairy P, while none had used milk from the other dairy. The Health Officer, who was the older of the two physicians in North Branch, then called attention to the fact that in his 17 years' experience in the town there had been no native typhoid for the first 12 while, during the remaining 5, native cases had occurred in small scattered numbers at considerable and irregular intervals. A list of these cases was compared with the lists of customers of Dairy P. It developed that the family maintaining Dairy P had arrived in North Branch and engaged in the milk business just 5 years before and that, of the 21 native cases, 17 were definite customers, 2 were occasional or indirect customers, while the remaining 2 may have been customers. The other dairy had not had any of the infected families as customers.

---

[96] Pub. Health Rpts. 37. Part 4. P. 2942. 1922.

[97] Am. Jr. Pub. Health. 4. P. 667. 1914.

The evidence suggested a carrier connected with Dairy P. The family consisted of the father and mother, two sons, a daughter and a grandchild; hired help was not employed and the only visitor was another daughter. There was no history of recent typhoid, but the father, mother and two daughters had been ill with it 22 years before. No one but the sons handled the milk, although the mother washed the cans; she maintained that she always used the water scalding hot. The mother showed a positive Widal but the urine and feces were negative and additional tests were not made.

Subsequent events completed the evidence. The family closed up the business and moved away within a month of the investigation and then, during the next five years, not a case of native typhoid developed. It thus appears that the native typhoid in the town of North Branch during a period of five years resulted from a carrier connected with a milk supply. The intermittency of the infection may have been due to only an occasional elimination of the organisms and to the careful methods, including the use of scalding water, practiced by the mother in washing the cans.

Ex. 6. The dangers of typhoid carriers in connection with single cow dairies is evident from an experience reported by Feezer.[98] Early in 1916 several cases of typhoid appeared in a town having a population of 12,000. Three cases had secured milk from Mrs. H. B., who kept one cow and sold a part of the milk. Mrs. H. B. gave no history of typhoid. In June she gave a positive Widal and was ordered to discontinue the sale of milk. This order was ignored and a second series of cases, appearing in October, was again traced to milk sold by Mrs. H. B. The State Board of Health then ordered a discontinuance of the sale of milk and the cow was disposed of. Early in 1918 Mrs. H. B. again sold milk occasionally and a family newly arrived in town took milk from this source; two cases of typhoid appeared in the family. Mrs. H. B. was thus apparently responsible for three distinct groups of typhoid cases.

Ex. 7. In Denver, Colorado,[99] 40 cases of typhoid appeared between February 26 and March 14, 25 of which used milk from one dairy and 13 from another; the second dairy sold raw milk to the first and was, therefore, directly or indirectly involved in 38 of the cases. The milk came from a ranch where there had been typhoid some years before and where the manager had the disease in January. Milk from the same ranch sold to a dairy that pasteurized, caused no typhoid.

---

[98] Am. Jr. Pub. Health. 8. P. 871. 1918.

[99] J. A. M. A. 86. P. 1219. 1926.

Ex. 8. One of the very extensive milk-borne typhoid epidemics occurred in Montreal, in 1927, and data with reference to it have been reported by Lumsden, Leahe, Crohurst and Waller.[100] From March 1 to July 16 there were 5,014 cases and 488 deaths. Two peaks occurred in the epidemic, one about the middle of March and the other about the middle of May. The water supply was excluded because the distribution of cases was unrelated to the two separate and distinct supplies and also because Verdun, an adjoining city with a separate water supply, had many cases. Milk was accepted as the cause of the outbreak because of the age distribution of the cases, 903 being between 0 and 10 years and 718 between 10 and 20 years, and because a large percentage of the cases used the products of Dairy M.D.C. In the institutions, such as boarding houses, orphanages and homes for helpless adults, there was a high incidence among the inmates when milk from Dairy M.D.C. was used but a normal rate when the milk came from other dairies. The supply distributed by Dairy M.D.C. came from 1,200 to 1,500 farms and it was estimated that this milk was exposed more or less to a population of 20,000. Some of it came through four receiving stations, one of which used water for washing from a polluted river. At the city plant the milk was pasteurized in a machine of an efficient type but as it was operated there appeared to be some leakage from the pasteurizer before the pasteurization was complete. The plant foreman was found to be a carrier and was relieved from duty on April 16. He, accordingly, could not have been the cause of the cases occurring during the second peak of the epidemic. It seemed probable that a portion of the raw milk coming to the plant was distributed without being pasteurized since, at certain times, more raw milk was delivered than was run through the pasteurizing machine.

Examples of butter-borne typhoid. Ex. 1. A typhoid epidemic occurred at Anoka City, Minnesota,[101] which was eventually traced to butter. Cases of typhoid were developing and the Health Officer had exonerated a suspected milk supply. The population included numerous persons previously ill with typhoid so that a carrier was a probability. After a search for the main route of infection had eliminated public water supplies, wells, flies, milk and contact, it was noted that most of the cases occurred in one ward and all dealt with one grocer so that a food infection seemed probable. Eventually a farm was located from which butter was sold to the grocer during the illness and convalescence of an unreported case of walking typhoid in a hired girl. At the time of the report between 35 and 40 cases with 6 deaths had occurred.

---

[100] Am. Jr. Pub. Health. 17. P. 783. 1927.

[101] Minn. State Bd. Health. 4th Bien. Rpt. P. 240. 1913.

Ex. 2. Boyd[102] reported the following history indicating that a small typhoid epidemic was spread through butter.

Township X, in northeastern Iowa, is essentially rural with one village in which a creamery is located. Between August 12 and 21, seven cases of typhoid developed in the township and September 2 another case was recognized. Two cases lived in the village while the others were widely scattered in the country; two of the country families had two cases each.

None of the patients had been away from home, except on trading trips to towns that were free from typhoid. There seemed to be no opportunity for infection through contact or through food or drink consumed away from home and the only food used at the probable time of infection was butter produced in the creamery located in the village; all the cases were considered the heavy butter consumers of their respective families and five were also heavy consumers of buttermilk from the same creamery. Including the patients, 29 persons lived in the six houses invaded.

On a farm in an adjoining township there existed a case of typhoid, the patient having gone to bed July 31. Cream from the premises was sold to the creamery in Township X until August 23. In the early stages of the illness the body discharges from the case were not properly disposed of; it was reported that undisinfected feces stood out of doors, accessible to flies. Four cases developed on the premises and three others in the immediate neighborhood.

In the creamery the cream was not pasteurized before ripening and churning. Most of the butter made was shipped to New York and only a small amount was distributed locally to creamery patrons and to stores in the village.

Example of cheese-borne typhoid. A typhoid epidemic due to cheese has been recorded by Rich.[103] Fifty-one cases occurred in and around the village of Bath, Michigan, from May 25 to July 11, 1917, and resulted in four deaths. On June 6 the Health Officer requested aid of the State Department of Health, but an investigation showed

[102] J. A. M. A. 69. P. 2030. 1917.

[103] Am. Jr. Pub. Health. 13. P. 210. 1923.

no common cause and nothing further was done until, on account of the increasing cases, an extensive investigation was begun on June 22. On July 5 several cases were reported from Ypsilanti and Lansing which subsequent study showed were connected with the Bath epidemic.

Water and milk were quickly eliminated. In considering the three recent public gatherings it was found that, of 16 persons attending the first, 6 contracted typhoid, of 13 attending the second, 3 developed the disease, while a list of those attending the third could not be secured; a considerable variety of foods was served at these gatherings. Ice cream was at one time suspected of causing the epidemic, but the manufacturing company shipped to over 100 cities and villages in which no typhoid could be traced to it and not all of the cases ate ice cream within the critical time.

Cheese was suggested as a cause of the epidemic by the wife of one case and the mother of another, and investigation showed that nearly all the primary cases had eaten cheese during the probable infection period. Cases were then located which did not live in Bath but which had eaten cheese secured there. The cheese sold in Bath during the period of infection was traced through the wholesale house and it was found that cheese from the same source appeared to be responsible for typhoid in Ypsilanti and Lansing. A study of conditions in the plant where the cheese was made showed that, while the cheesemaker had never had typhoid and gave a negative Widal, a person giving a positive Widal worked for a farmer sending milk to the factory and another giving a doubtful Widal worked on a second farm supplying milk for cheesemaking. Records of the State Department of Health showed 23 cases of typhoid within a few years in the vicinity of the town in which the factory was located.

Some of the cheese involved was sold comparatively soon after its manufacture, the shortest time between the making and the retail sale being only about nine days. Longer curing might have reduced or perhaps eliminated the infection. Inquiry revealed that no typhoid developed from the cheese that was shipped outside the state; the increased age of this cheese may have resulted in the death of the typhoid organisms that were presumably present.

One of the interesting points in connection with the epidemic was the incubation period which varied from 14 to 37 days.

Example of ice-cream-borne typhoid. An extensive ice-cream-borne typhoid epidemic has been reported from Pennsylvania.[104] A total of 1,851 cases involving 21 counties were investigated. Ice cream manufactured by the H Creamery Company, or ice cream made from cream supplied by that concern, appeared to be the common medium of infection in 70.2 per cent of the cases and there was an additional 6.7 per cent with which the ice cream was probably the cause, giving a total of 76.9 per cent. In a number of instances retailers handled the H product at odd times or without knowing the source of their supply so it is reasonable to suppose that a still larger percentage of the cases was due to this source of infection. Probably not all of the cases were discovered. The investigation of a typhoid epidemic in Virginia showed that a majority of the patients had eaten ice cream made from cream secured from the H Company, and if a study had been made of the typhoid in other states additional evidence might have been found.

---

[104] Pa. Com. of Health. 11th An. Rpt. P. 241. 1916.

Investigations of 1,775 dairy farms gave histories of illness in 17 instances but specimens could not be obtained from each suspected individual and those that were secured failed to show typhoid organisms. The creameries and stations through which the cream passed before reaching the H Company yielded no definite clue as to the source of the infection. Information concerning dairy farms in other states which supplied cream was not secured. Early in the study of the epidemic, specimens were obtained from each employee of the H Company who had anything to do with handling the ice cream or pasteurized cream but yielded only negative results. Although it is possible that butter manufactured by the H Creamery may have been a factor in the epidemic, there was no evidence of this.

Importance of milk as a cause of typhoid fever. With the abundant evidence of the spread of typhoid fever through milk the percentage of the total cases that are due to it becomes of importance. Calculations have been made by estimating the total cases from the mortality statistics and tabulating the cases due to dairy products from reports of the epidemics appearing in the literature, but the percentages so obtained must be entirely too low since not all of the cases due to dairy products are recognized and certain of those that are recognized are not reported. Data have also been secured by determining, as accurately as possible, the source of infection for the cases appearing in a given area during a certain period. Various investigations have been carried out along this line in the District of Columbia and the cases in which the infection certainly, or almost certainly, was contracted in the District have been summarized by Lumsden and Anderson[105] as follows:

---

[105] U. S. Pub. Health. Mar.-Hosp. Serv. Hyg. Lab. Bul. 78. 1911.

SOURCES OF THE TYPHOID CONTRACTED IN THE DISTRICT OF COLUMBIA

	1906 (June 1 to Nov. 1)		1907 (May 1 to Nov. 1)		1908 (May 1 to Nov. 1)		1909	
	Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent
Infection attributed to milk..	85	11.30	48	9.18	52	9.59	13	2.29
Infection attributed to contact	54	7.30	102	19.50	114	21.03	69	12.19
Infection attributed to private well water..............	....	......	....	......	....	......	7	1.23
Accounted for..............	139	18.60	150	28.68	166	30.62	89	15.72
Unaccounted for............	608	81.40	373	71.32	376	69.38	477	84.28
Total.................	747	100	523	100	542	100	566	100

These results show that, in the various periods covered, from 2.29 to 11.30 per cent of the typhoid was considered to be milk-borne; the cases attributed to milk comprised only those which occurred in the course of pronounced outbreaks among the consumers of certain supplies and it seems very probable that some of the cases caused by milk-borne infection were missed and thus included with the cases unaccounted for.

The Massachusetts Department of Public Health[106] has reported data on the importance of milk in spreading typhoid that are of special interest because of the length of the period covered and the number of cases included; these may be summarized as follows:

IMPORTANCE OF MILK AS A CAUSE OF TYPHOID IN MASSACHUSETTS

Period	Cases of Typhoid Fever			Typhoid Outbreaks Traced to Milk		
	Reported	Traced to Milk		Number	Average Number per Year	Per cent of Outbreaks of all Diseases Traced to Milk
		Number	Per cent			
1907–14	23,482	2,215	9.43	50	6.25	69.45
1915–18	6,331	496	7.83	16	4.00	57.14
1919–23	4,105	297	7.23	12	2.40	70.59
1924–26	1,705	81	4.10	7	2.33	77.78

---

[106] J. A. M. A. 67. P. 1997. 1916: Am. Jr. Pub. Health. 10. P. 66. 1920: Am. Jr. Pub. Health. 14. P. 963. 1924: Am. Jr. Pub. Health. 17. P. 1019. 1927.

The above findings indicate that there has been a general decrease in the percentage of the typhoid cases due to milk from 9.43 in the first period to 4.10 in the last. Although the length of the periods is not uniform, a decrease in the total cases of milk-borne typhoid is also evident.

In 1920 a part of the Massachusetts data [107] was reported on a yearly basis as follows:

IMPORTANCE OF MILK AS A CAUSE OF TYPHOID IN MASSACHUSETTS

Year	Cases of Typhoid Fever		
	Total	Traced to Milk	
		Number	Per cent
1910	3,452	537	15.5
1911	2,238	64	2.9
1912	2,088	17	0.8
1913	2,398	214	8.9
1914	2,333	223	9.6
1915	2,204	153	6.9
1916	1,515	166	10.8
1917	1,547	85	5.5
1918	1,065	92	8.6
1919 (9 mos.)	654	20	3.1
Totals	19,494	1,571	Av. 8.1

This tabulation shows that the general decrease in the percentage of the typhoid cases due to milk involves a great deal of fluctuation from year to year. Such a variation is to be expected because of the influence of one of the larger epidemics in markedly increasing the total milk-borne cases.

While the data reported for Massachusetts can not be assumed to represent conditions in other areas they are very suggestive. It seems probable that milk is becoming of less importance in the spread of typhoid because of improvements in the methods of production and handling and the increasing use of pasteurization. In certain of the larger cities milk-borne typhoid has now been practically eliminated. Epidemics in the smaller towns will undoubtedly continue until efficient pasteurization is more widely used than at present or some other preventive measure is instituted.

---

[107] See ref. 106.

In this connection the following tabulation prepared by Armstrong and Parran[108] and showing the recorded milk-borne outbreaks of all diseases in the United States by five-year periods from January 1, 1881, to January 1, 1927, is of interest.

RECORDED MILK-BORNE OUTBREAKS OF ALL DISEASES IN THE UNITED STATES

Year	Outbreaks	Year	Outbreaks
1881–85	3	1911–15	238
1886–90	14	1916–20	130
1891–95	26	1921–25	130
1896–1900	33	1926	12
1901–05	60		
1906–10	145	Total	791

These results show an increase in the reported milk-borne epidemics from the time the first epidemic was recognized until the period 1911–15, when a maximum was reached; a decrease then began. The detection of milk-borne epidemics probably has been more nearly complete during the period of the decrease than previously so the decrease is undoubtedly of significance.

The growth of the typhoid organism in milk. Although the nutrients provided by milk are adequate for the growth of *Eberthella typhosa,* multiplication under practical conditions is influenced by various factors, such as a low holding temperature, the other species present and the reaction of the milk. The effect of temperature is illustrated by the results secured by Brown,[109] who found that in sterile milk the organism increased rapidly at 36° C. (96.8° F.), somewhat more slowly at room temperature (from 21.7° to 25.6° C.—71° to 78° F.) while at storage temperature (from 5.6° to 6.7° C.—42° to 44° F.) the numbers decreased rapidly at first and then more slowly. The growth of the typhoid organism in raw milk is shown by various investigations of the germicidal property of milk and by the work of Eyre,[110] who noted an enormous increase in milk drawn aseptically.

[108] See ref. 92.

[109] Mass. State Bd. Health. 39th An. Rpt. P. 417. 1907.

[110] Jr. State Med. 12. P. 728. 1904.

The multiplication of *E. typhosa* in milk indicates that the addition of only a few organisms to a milk supply may be the cause of the infection of a considerable number of people and the extent of some of the milk-borne typhoid outbreaks reported suggests such growth. The influence of the temperature of holding milk appears to have been an important factor in explaining a milk-borne outbreak at Pullman, Washington.[111] Sixty cases of typhoid developed among the students at one boarding house while the same milk supply was used at two other boarding houses without causing illness; investigation showed that at the affected boarding house the milk was allowed to stand at room temperature all day while at the other two it was properly iced.

The influence of acid development in milk or cream on the typhoid organism deserves special consideration because of its relationship to various practical problems. Much of the cream used in the manufacture of butter is received at the plants in a sour condition and the possibility of living typhoid organisms being present is of importance. Sour cream is retailed in various cities to a certain class of trade and, because of the effect of acid and heat in combination in precipitating and hardening the casein, can not be pasteurized in a sour condition; dealers sometimes desire to sell raw cream which sours during transportation but can not do so if the regulations insist on pasteurization. The possibility of buttermilk spreading typhoid organisms must also be considered. It should be recognized that pathogens other than the typhoid organism may be present in acid materials and that *E. typhosa* constitutes only a part of the problem, but it has received most of the attention along this line because of the comparatively large number of typhoid epidemics due to dairy products.

The results of various investigations show definitely that as lactic acid is developed in milk *E. typhosa* is gradually killed. Krumwiede and Noble[112] found the rate of destruction in sour cream to be proportional to the degree of acidity and the number of typhoid organisms. They noted that, "With a moderate contamination the typhoid bacilli are killed in about four days. With a heavy contamination or where initial multiplication has taken place a longer time may be required." Heinemann[113] used a sterilized mixture of milk and lactic acid and found the limit of acid tolerance of *E. typhosa* to lie between 0.3 and 0.4 per cent; the organism was destroyed at about 0.42 per cent and there was a great reduction at 0.35 per cent. This investigator believes that acid tolerant strains apparently multiply in amounts of acid destroying the majority of cells. He also suggests the possibility of bacterial products other than acid playing a part in the destruction of such organisms as *E. typhosa.* Marsh[114] concluded that *E. typhosa* is usually destroyed in milk in a short time at about the acidity occurring in fresh buttermilk as obtained in the New York market and that buttermilk would seem to be free from danger as a means of distributing the typhoid, paratyphoid or dysentery (Flexner) organisms in the ordinary course of events. The investigations of Northrup[115] suggest that the acids produced by different organisms have a variable effect on *E. typhosa, L. bulgaricus* appearing to be rather ineffective in inhibiting it.

---

[111] Wash. State Dept. Health. 1st Bien. Rpt. P. 70. 1921–22.

[112] Bur. of Lab. City of N. Y. Col. Studies. 8. P. 313. 1914–15.

[113] Jr. Inf. Dis. 16. P. 479. 1915.

Although the acid developed in milk and cream undoubtedly often destroys the typhoid organism, it would be dangerous to depend on such acid to make various dairy products safe as far as this organism is concerned. When milk and cream sour slowly there is the possibility of considerable multiplication of the typhoid organism so that greater numbers may be present than when the same material was in a sweet condition.

Longevity of E. typhosa in dairy products. The early investigations on the longevity of *E. typhosa* in dairy products indicated that the organism may live for considerable periods and more recent studies carried out under conditions comparable to many present practices suggest the same thing. Brown[116] found the typhoid organism still alive in milk held at 5.6° to 6.7° C. (42° to 44° F.) after 22 days. Washburn[117] noted that in an ice chest the organism lived in milk for 43 days and in butter 151 days; it was actively motile in the milk for 20 days. In the studies reported by Berry[118] *E. typhosa* lived in milk at ice box temperature for 290 days and at room temperature for 187 days. Three strains of this organism were studied as to their survival in butter at ice box temperature; one lived 22 days or less, one 63 days and one 110 days. In ice cream stored in a hardening room which fluctuated from —22.2° to —13.3° C. (— 8° to 8° F.), Prucha and Brannon[119] found that there was a continual decrease in the number of inoculated typhoid organisms but that some were still alive after two years and four months of storage, when the examinations were terminated.

---

[114] Am. Jr. Pub. Health. 8. P. 590. 1918.

[115] Mich. Agr. Expt. Sta. Tech. Bul. 10. 1911.

[116] See ref. 109.

[117] U. S. D. A. B. A. I. 25th An. Rpt. P. 297. 1908.

[118] Jr. Prev. Med. 1. P. 429. 1927.

[119] Jr. Bact. 11. P. 27. 1926.

From the above results it is evident that typhoid organisms can live in dairy products for extended periods and that long holding can not be expected to make these materials safe.

Sources of E. typhosa in milk. The sources from which *E. typhosa* most commonly gets into milk are shown by the following summary, prepared by Armstrong and Parran,[120] of 373 outbreaks for which the means of milk infection were designated:

Sources from which Milk was Infected with Typhoid Organisms

Probable Source of Infection	Number of Outbreaks
Carrier (farm, distributing plant, etc.)	162
Active case (farm, distributing plant, etc.)	134
Exchange of bottles from homes with infection	37
Use of polluted water on utensils, etc	28
Soilage of cows in polluted water	4
Miscellaneous (intermediate persons, etc.)	8

These results indicate that carriers and active cases coming in contact with milk supplies have been responsible for the great majority of the milk-borne typhoid outbreaks. The exchange of bottles from homes in which there are cases and the use of polluted water on utensils are also of considerable significance. The tabulation of Trask [121] likewise shows the great importance of direct contamination from human sources in infecting milk with the typhoid organism.

An instance in which milk was involved in an epidemic that was primarily water-borne has been reported by Halliday and Beck.[122] Among a total of 620 cases, 200 were due to contaminated milk; the milk was probably infected by a person who acquired the infection from the water.

Relationship of typhoid carriers to the infection of milk. Typhoid carriers are probably becoming of comparatively greater importance as a source of the typhoid organism in milk because of the control of such other sources as water, flies, etc. The carrier problem is complicated by the fact that carriers are of more than one type. Some have definitely had typhoid and on recovery continue to eliminate the organisms for more than the usual period, while others may not have been ill, or at least not seriously so, but the organisms nevertheless have become established in the body; errors in diagnosis also result in carriers giving no history of typhoid.

---

[120] See ref. 92.

[121] U. S. Public Health Mar.-Hosp. Serv. Hyg. Lab. Bul. 56. P. 25. 1909.

[122] Jr. Prev. Med. 2. P. 49. 1928.

Carriers may eliminate typhoid organisms long after the period of illness. The following data tabulated by Armstrong and Parran [123] show the duration of the carrier state (years from date of typhoid attack to date of causing outbreak) in 41 milk-borne outbreaks for which this information was secured.

DURATION OF CARRIER STATE WITH CARRIERS CAUSING MILK-BORNE TYPHOID EPIDEMICS

	Number of Epidemics
Under 1 year	10
2 to 10 years	13
11 to 20 years	8
21 to 30 years	5
31 to 40 years	1
41 to 50 years	3
51 to 60 years	1

The prevalence of permanent carriers among recovered typhoid patients varies widely, according to the results of different studies. In a review of the literature Leach, Dehler and Havens [124] noted percentages from 0.5 to 11.6 per cent; these investigators found that 10.3 per cent of 156 individuals, who had recovered from typhoid within from six months to two years, were carriers.

Welch, Dehler and Havens [125] studied the feces and urine of 1.076 healthy persons, including 1,004 men and 72 women, employed in the dairy industry in Alabama; both feces and urine were examined an average of 2.3 times. Thirty-nine typhoid carriers, 13 paratyphoid A carriers, and 3 paratyphoid B carriers were found, making a total of 55 or 5.1 per cent. Three hundred persons in a community where,

[123] See ref. 92.

[124] Am. Jr. Pub. Health. 16. P. 391. 1926.

[125] J. A. M. A. 85. P. 1036. 1925.

until a few years ago, there was a high typhoid rate yielded 31 carriers. If these are excluded there were still 24 carriers or 3.1 per cent among 776 persons. The incidence of fecal and urinary carriers was as follows: fecal typhoid 21; urinary typhoid 18; fecal paratyphoid A 8; urinary paratyphoid A 5; fecal paratyphoid B 3; urinary paratyphoid B 0. Of the 55 carriers 34 were found on the first examination so that subsequent tests detected 21 that would have been missed with only one examination. The importance of the personal habits of a carrier in preventing the infection of other persons was shown by two urinary carriers who owned and operated a dairy to which no case of typhoid could be attributed over a period of at least five years.

The detection of a carrier among the persons coming in contact with a milk supply is often easy but sometimes difficult. The first step is the use of the Widal test because of the ease with which it can be applied. Any person giving a positive Widal, especially if there is a history of typhoid, is to be looked upon with suspicion. When a positive Widal is secured it is desirable that the excreta be examined for *E. typhosa* but in the study of certain epidemics this has not been done. An occasional carrier fails to give a positive Widal and such individuals can be located only through examinations of the excreta. In all cases such examinations should be made more than once when negative results are secured because of the intermittent elimination of the organisms. In some instances the infection of dairy products has occurred through carriers that could not be detected by the Widal test and such persons greatly complicate the location of the sources of infection in milk-borne epidemics.

PARATYPHOID FEVER

The spread of paratyphoid fever through dairy products shows the same general features as the spread of typhoid. Although only a comparatively few epidemics have been reported, the number is large enough to indicate the possibilities along this line.

Armstrong and Parran[126] have collected records of seven paratyphoid epidemics due to dairy products that occurred in the United States up to January 1, 1927. Based on incomplete data these involved 434 cases with 15 deaths, the largest epidemic including 110 cases with 12 deaths. Five of the outbreaks were due to milk and two to ice cream; the milk was raw in three instances, in one it was bulk pasteurized milk, which was probably infected in a cafeteria, and in one it was certified milk.

---

[126] See ref. 92.

Examples of milk-borne paratyphoid. Ex. 1. Wade and McDaniel[127] have reported a paratyphoid outbreak that occurred at the University of Minnesota among the students, faculty members, cafeteria employees and other persons that had eaten one or more meals at the Men's Union cafeteria; 106 cases with 2 deaths were reported and 103 cases were patrons or employees at the cafeteria. The diagnosis was confirmed through agglutination tests with blood from 92 patients and through the isolation of paratyphoid B from stools or urine in 21 cases and from the blood in 1 case.

In the investigation various food products were ruled out and finally bulk pasteurized milk appeared to be responsible. This suspicion was substantiated by cases in two children who, with their parents, took only Sunday dinners at the cafeteria and who became ill following a meal at which they ate the same food as the parents with bulk milk in addition. Since the bulk milk sold at the cafeteria constituted only 1.22 per cent of the dealer's output of bulk milk, infection at the cafeteria seemed probable.

Four cafeteria employees were found to be carrying paratyphoid organisms and evidence was secured indicating the probable infection of other employees and patrons.

Ex. 2. An outbreak of paratyphoid that involved largely infants and young children occurred in New Rochelle, New York, and nine neighboring communities and has been reported by Williams.[128] The epidemic was due to a milker on a certified dairy who was found to be a carrier of paratyphoid B.

The investigation included 50 case histories, the information being secured either from the family physician or the parents. The characteristic symptoms were a green, mucoid, blood-tinged diarrhea, with fever, abdominal pain and occasional vomiting. The youngest patient was three months old, while 49 of the 50 patients were under six years; the remaining patient, 40 years of age, was on a special diet which included milk from the certified dairy.

Each of the cases was known to have used the milk in question and there were no similar cases at the time among persons using any other milk. Water was excluded because the area involved was served by a number of separate supplies. The clue to the correct diagnosis of the outbreak was the isolation of paratyphoid B from the stool of one of the children during convalescence. The organism was then secured from three other children. Paratyphoid B was isolated from the stool of the carrier, who was a milker on the farm producing the milk, and also from the stool of his four year old child. The last raw certified milk from the dairy in question was sold on May 7 and the last case reported became ill on May 13.

---

[127] J. A. M. A. 83. P. 1416. 1924.

[128] J. A. M. A. 84. P. 251. 1925.

Growth of paratyphoid organisms in milk. The paratyphoid organisms develop rapidly in milk. Brown[129] found that a paratyphoid strain isolated from the gall bladder of a man grew luxuriantly in milk at either 36° C. (96.8° F.) or room temperature (21.7° to 25.6° C.—71° to 78° F.), although more rapidly at the former than at the latter temperature.

Longevity of paratyphoid organisms in dairy products. The paratyphoid organisms seem to be capable of retaining their vitality in dairy products for unusually long periods. At ice box temperature Berry[130] found paratyphoid A lived in milk for 107 days and in butter 117 days while paratyphoid B was alive in milk after 324 days, which was as long as the examinations were made, and in butter after 212 days.

Sources of paratyphoid organisms in milk. Of the seven outbreaks of milk-borne paratyphoid tabulated by Armstrong and Parran,[131] three were due to carriers, two to active cases on the producing farms, in one the source of the organism was not determined and in one no statement with reference to the source was made in the report.

DYSENTERY AND DIARRHEA

The general characters and methods of distribution of the various organisms responsible for dysentery and diarrhea suggest that these diseases might be spread through dairy products and this has been substantiated by the occasional reporting of such outbreaks. Records of six epidemics that occurred in the United States up to January 1, 1927, have been summarized by Armstrong and Parran.[132] On the basis of incomplete data 92 cases and 5 deaths were involved in these outbreaks. Milk was responsible in each instance; in three epidemics the milk was raw while in the other three the nature of the supply was not stated.

Example of milk-borne dysentery. An epidemic of dysentery occurred at the New England Deaconess's Home in Haverhill, Massachusetts,[133] and involved about 20 cases among an exposed group of 75 persons. Food was excluded through the occurrence of cases outside the Home that had not partaken of the common diet and water was excluded because of the outside cases and also because at least 90 per cent of the cases were in children, which would not have been the situation had water been involved. All of the children were regular consumers of milk which came from Farm R. This farm had good sanitary arrangements but the cans were washed at another farm where conditions were unsatisfactory. Cases of illness occurred at Farm R before the onset of the cases at the Home and involved the father and other members of the household. The father continued to milk during the entire course of the illness.

---

[129] See ref. 109.

[130] See ref. 118.

[131] See ref. 92.

[132] See ref. 92.

Growth of dysentery organisms in milk. The dysentery organisms grow well in milk. Brown [134] found the Shiga type developed at both 36° C. (96.8° F.) and room temperature (21.7° to 25.6° C.—71° to 78° F.) but more rapidly at the former than at the latter temperature.

Longevity of dysentery organisms in dairy products. Berry [135] found that the dysentery organisms (Flexner and Shiga) were comparatively short lived in butter and disappeared in from 7 to 18 days at ice box temperature.

Sources of dysentery and diarrhea organisms in milk. In the six milk-borne epidemics of dysentery and diarrhea summarized by Armstrong and Parran [136] the causative organisms were attributed to cases on the farms or at the dairy in three, while in the other three the sources of the organisms were not stated.

SEPTIC SORE THROAT

Although outbreaks of milk-borne septic sore throat have been recognized in Europe for many years, comparatively little attention was given them in the United States until 1912 when serious epidemics occurred in several cities. Milk-borne septic sore throat is included in the group of diseases with which the infectious material comes primarily from man but it should be noted that in a number of instances the milk appears to have been infected from abnormal udders; in these cases, however, the types of organisms suggest that the udders were infected from human sources, probably by the transfer of organisms from an infected throat to the hands and then to the udders through the teat canals.

---

[133] Mass. State Dept. Health. 2nd An. Rpt. P. 486. 1916.

[134] See ref. 109.

[135] See ref. 118.

[136] See ref. 92.

Septic sore throat is the one disease with which epidemics are very largely milk-borne. Many cases are, of course, due to contact but definite epidemics commonly include cases with more or less characteristic symptoms and quite regularly involve milk supplies. These outbreaks are frequently extensive and, accordingly, attract a great deal of attention; a number of those that have occurred in the United States have each involved more than 1,000 cases while an outbreak in Chicago was estimated to include more than 10,000. The mortality is usually low, with the deaths occurring principally among old people as a result of complications. Armstrong and Parran[137] have collected from the literature data on 42 outbreaks of septic sore throat, due to dairy products, that occurred in the United States from 1908 to January 1, 1927; these involved, on the basis of incomplete data, 21,045 cases and 139 deaths. Milk was responsible for 41 of the epidemics and ice cream for 1; the milk was raw in 19, pasteurized in 3 and certified in 1, while in 18 the character was not stated.

Examples of milk-borne septic sore throat. Ex. 1. An outbreak of milk-borne septic sore throat that occurred in Boston and vicinity, in 1911, has been reported by Winslow.[138] There were two definite foci centering around Boston and Marlboro, respectively.

The Boston epidemic involved the Back Bay and other sections of Boston, the town of Brookline and the city of Cambridge and records of 1,043 cases in this vicinity were obtained. There was a sharply defined epidemic which began May 8, rose to a maximum May 14 and practically ceased May 22. Cases were concentrated in the affected families, 22 per cent having three or more cases while only 56 per cent had but one case. Adults suffered more in proportion than children and there were about twice as many cases in females as in males. Forty-eight deaths were attributed to the epidemic. The distribution of the epidemic coincided with that of one of the two main milk supplies of the D Company. Cases appeared in Boston, Brookline and Cambridge where this milk was used and also in Marlboro and Southboro, the only other towns to which it was distributed. Over 85 per cent of the cases were on the delivery lists of the company and an additional 8 per cent, although not listed as subscribers, were reported to have used the milk. The D Company furnished about 1 per cent of the Boston milk and 2 per cent of the Cambridge supply. In Brookline not merely the epidemic condition but all tonsilitis as well was included and 65 per cent of these cases appeared on the delivery lists while 8 per cent more were reported to have used the milk; the company supplied about 7 per cent of the Brookline milk. A study of delivery lists for Cambridge and Brookline, where the records were fairly complete, showed that one family out of every four supplied had been affected.

---

[137] See ref. 92.

[138] Jr. Inf. Dis. 10. P. 73. 1912.

The outbreak in the Marlboro region seemed at first to complicate the situation. The disease was of the same type as in Boston. Records of 392 cases in the towns of Hudson, Marlboro and Southboro were secured. The household incidence was less marked than in Boston, over 83 per cent of the households having but one case and only 6 per cent having three or more; there was a slight excess of cases among males. One death, probably attributable to the epidemic, occurred in Hudson. Instead of a sudden outbreak, the disease was present all through April and May and was evidently spreading from person to person. In Hudson, where no D milk was sold, the case distribution was fairly even over a period of five weeks, while in Marlboro and Southboro in addition to the general prevalence over the whole period there was an outbreak simultaneous with that in Boston. During the outbreak period the excess of cases was among the users of D milk.

The findings suggest that the sore throat prevailed in prosodemic form in Hudson, Marlboro and Southboro during April and May and then, in the second week in May, appeared as a sharp epidemic following a definite milk supply in Boston, Brookline, Cambridge, Marlboro and Southboro. The milk involved in the spread of the sore throat was secured from farms in the Marlboro, Southboro and the adjacent sections and may have been infected from human cases known to be numerous in the neighborhood. No record was secured of any well defined case that came in direct contact with the milk but cases occurred in families of employees of the D Company. No cattle diseases were found on any of the farms.

The D milk had an excellent reputation and many precautions were taken in its production. Farms and cattle were regularly inspected by a veterinarian, facilities for handling the milk were the best and the whole process was controlled through frequent bacteriological analyses. The use of milk from cows having udder abnormalities of any description was forbidden and the farmers understood that when a contagious disease in persons occurred on the farms it was to be reported to the D Company at once and the milk would be paid for but not used.

Ex. 2. An outbreak of epidemic sore throat occurred in Chicago in December, 1911, and January and February, 1912. Capps and Miller[139] studied its relationship to the milk supply, while Davis[140] investigated the causative organism. It was conservatively estimated that over 10,000 cases were involved.

The physicians attending the cases agreed that the epidemic was quite different than the ordinary tonsilitis. After milk was suggested as a cause, an investigation showed that most of the cases were consumers of milk from Dairy X. Of a series of 173 cases selected because of their severity and conformity to the epidemic type of throat infection, 147 or 85 per cent were users of milk from Dairy X at the time of the illness; 15 or 79 per cent of the 19 fatal cases used milk from this source. In the districts investigated Dairy X supplied about 10 or 12 per cent of the milk. Of the households using milk from Dairy X, 51 per cent were affected as contrasted with 7.2 per cent of those using other milk. The average morbidity for each affected household using milk from Dairy X was 2.3 persons, while for the affected households supplied with other milk it was 1.2 persons. The ratio of morbidity among users of milk from Dairy X to the morbidity among users of other milk was accordingly about 14 to 1. The incidence of the disease was studied among the nurses in hospitals; the total number of nurses using milk from Dairy X was 153 and 80 or 52 per cent of these were attacked, while the total number using milk from other dairies was 721 and of these 35 or 4.8 per cent were attacked. At one of the hospitals the 70 occupants of the children's wards passed through the epidemic without a case of sore throat, while among the nurses and internes there were many cases. All of the milk for the hospital came from Dairy X but the milk for the children was delivered raw and pasteurized at the hospital, while the remainder of the milk came from the usual pasteurized supply. At Batavia, Illinois, which was the center of the Dairy X herds and where the milk was pasteurized, it was found that there was a great deal of sore throat, not only among the customers of Dairy X but also among the customers of other dairies.

Sore throats of the epidemic type were present on 11 farms supplying Dairy X, with a total of 29 cases. On 9 of these farms there was also mastitis among the cows. One cow had a chronic abscess of a hind quarter and, while the milk from this was thrown away, that from the other quarters was used; a girl on the same farm was very ill with sore throat. On another farm milk from a cow having typical mastitis of five weeks' duration showed streptococci, and streptococci were also secured from a girl who had been ill with sore throat five weeks before. Both the human and bovine cultures were fatal to guinea pigs and rabbits. On a third farm all three milkers had sore throats and there were 11 cases of mastitis among the cows. The milk sold by Dairy X was flash pasteurized, supposedly at a temperature of 71.1° C. (160° F.). Fluctuations in the temperature occurred, however, with a minimum of 54.4° C. (130° F.) on several days. There were pronounced failures of the pasteurization on December 17, 19, 28, 30 and on January 7 and 11 and explosive outbreaks of cases seemed to rather closely follow these dates. The final responsibility for the epidemic undoubtedly rests on the inadequate pasteurization.

---

[139] J. A. M. A. 58. P. 1848. 1912.

[140] J. A. M. A. 58. P. 1852. 1912.

Ex. 3. An epidemic of septic sore throat at Rockville Centre, Long Island, during June, 1914, was studied by Krumwiede and Valentine.[141] It was soon evident that the milk supply was the common element among the infected persons. The milk came from Dairy O, housing 22 cows, but the dairy also obtained some milk from two other small farms, R and C, and distributed about 400 quarts per day to 173 customers. Of the 232 cases, 205 secured their milk from this source. Other dairies delivered about 1,200 quarts per day, so in this epidemic a dairy distributing about 25 per cent of the milk had at least 90 per cent of the cases among its consumers.

At Dairy O, Miss W., daughter of the owner, developed sore throat on April 16. Mrs. W., the owner, developed sore throat on May 9, but claimed that she remained away from the dairy until May 28. On May 11 the driver developed a sore throat and on June 9 the milker was affected. Mrs. W. and the milker did most of the milking but were assisted at times by other persons, especially the driver.

Isolated cases of sore throat appeared among the users of the milk from Dairy O on April 21 and 27 and on May 17 and 21. Two cases occurred on May 30 in a family using milk from their own cow. From June 1 to 5 the number increased and then the main part of the outbreak occurred between June 6 and 14.

The bacteriological studies involved moist swabs from the throat of each individual on the three farms and from a number of the cases, a culture from a complicating peritonitis and milk from each quarter of all the cows. The samples from five cows showed a moderate number of streptococci in one or more quarters; one of these cows showed evidence of mastitis on physical examination, and another, Cow 21, showed a moderate number of streptococci in the milk from two quarters and an enormous number in that from another quarter giving a flocculent secretion. The streptococci from the three quarters of Cow 21 were hemolytic.

---

[141] Jr. Med. Res. 33. P. 231. 1915.

The throat cultures from the cases and from certain of the persons at Dairy O yielded hemolytic streptococci which were similar culturally to the organism from Cow 21 and to the one from the case of peritonitis. A hemolyzing organism was also secured from a person on Farm R but differed from the others culturally and morphologically. Serum from immunized rabbits used for agglutinations confirmed the relationship of the organisms of the main type, but these differed agglutinatively from organisms secured in other outbreaks of septic sore throat.

The following sequence of events indicates what probably occurred: Miss W. ill, April 16, with sore throat; contact infection of Mrs. W., May 9; contact infection of the driver, May 11; infection of Cow 21, probably from Mrs. W.; multiplication of the streptococci in the udder, resulting in the infection of the mixed milk and then in the outbreak. The infection thus appears to be of human instead of bovine origin. The cow showing mastitis on physical examination yielded a bovine type of Streptococcus.

Ex. 4. A sore throat epidemic occurred in Galesville, Wisconsin, in 1917, and was studied by Rosenow and Hess [142] and also by Henika and Thompson.[143] In a population of 941, there were 325 cases (34.5%) and 9 deaths (one report gives 11); 184 females and 141 males were involved. There were 242 families in the village and 121 were affected. The incubation period was 48 hours, as shown by the persons who drank milk or ate ice cream only once. Forty-eight persons using the milk escaped. The dairy responsible for the trouble was of the better type. Among 26 cows, 3 showed mastitis in one quarter and the milk of 2 of these appeared watery, while that of the third seemed normal. The milk of 6 cows, including the 3 with mastitis, showed enormous numbers of streptococci. The incriminated milk contained streptococci identical in appearance with organisms from the throats of patients and it was definitely shown that the organisms in the milk and in the throats were the

[142] J. A. M. A. 68. P. 1305. 1917.

[143] J. A. M. A. 68. P. 1307. 1917.

same. Three cubic centimeters of milk from an infected quarter killed a rabbit in about 12 hours and a salt solution suspension of swabs from the throats of patients did much the same thing. The same materials also caused rapid death in mice into which they were injected. Two mice, whose throats were swabbed once and which were fed bread soaked in the infected material, died; one of the animals showed large numbers of streptococci in the blood and pericardial fluid. Virulent organisms were found to be present in the udders of cows with no demonstrable disease. A temperature of 60° C. (140° F.) killed the organism, the injected animals remaining well.

Ex. 5. A milk-borne epidemic of septic sore throat in Portland, Oregon, which involved 487 cases with 22 deaths, has been reported by Benson and Sears.[144] The epidemic was checked by prompt detection of the responsible case and by heating the milk.

A child, aged two years, died March 25, 1922, with the clinical picture of sore throat and acute encephalitis. Other cases occurred on this and the next two days, all apparently referable to the milk of a single raw milk dairy. Several cases appeared in a hospital and many in a home for girls. The Health Bureau then ordered the heating of the milk from the dairy in question and within 24 hours the incidence of new cases declined to such an extent that the few which appeared could be ascribed to contact infection.

The cows were well housed in a wooden structure with concrete floors and proper facilities for flushing floors and handling excreta. The city milk inspection had always given the dairy one of the best ratings in the city as regards bacterial count and general quality of the milk. It was found, however, that cows having diseased udders were kept in the regular stalls and in some instances only the milk from the diseased quarter was excluded from the milking. The milkers did not always handle the same cows so that milk which it was intended to exclude might have been included.

Similar hemolytic streptococci were isolated from the throats of numerous patients, from the throat of one of the milkers who had large inflamed tonsils and a mild sore throat and from one quarter of the udder of one of the cows; these were of the human type. From a number of other cows hemolytic streptococci were also isolated but these appeared to be of the bovine type. It seems probable that the milker in question infected the udder of the cow, producing mastitis, and that on one or more occasions milk from this animal was included with that distributed.

[144] J. A. M. A. 80. P. 1608. 1923.

An investigation showed that, of the 487 cases, 166 were severe, because of serious complications or protracted sepsis, while 321 were moderate or mild. Among 69 users of the milk in a hospital there occurred 23 cases with 3 deaths and among 104 cases in a girls' home there were 45 cases with no fatalities; all the remaining cases were in private homes. Several persons became infected by drinking the milk in the homes of regular customers and among these 2 deaths occurred.

Importance of milk as a cause of septic sore throat. The importance of milk in spreading septic sore throat is shown by the data secured in Massachusetts;[145] these may be summarized as follows:

IMPORTANCE OF MILK AS A CAUSE OF SEPTIC SORE THROAT IN MASSACHUSETTS

Period	Cases of Septic Sore Throat			Septic Sore Throat Outbreaks Traced to Milk		
	Reported	Traced to Milk		Number	Average Number per Year	Per cent of Outbreaks of All Diseases Traced to Milk
		Number	Per cent			
1907–14	....	2,512	....	7	0.87	9.72
1915–18	1,401	867	61.88	7	1.75	25.00
1919–23	829	118	14.23	3	0.60	17.65
1924–26	415	0	0.00	0	0.00	0.00

These results show that in Massachusetts there has been a decided decrease in the reported cases of septic sore throat and that milk has become of less importance as a cause of the cases that do occur.

Organisms responsible for epidemics of septic sore throat. From the investigations carried out it appears that the organisms responsible for septic sore throat are hemolytic streptococci of various types.[146] The milk may be infected either directly from human sources or from the udders of the producing cows but with either method the organisms represent essentially human types as distinguished from bovine types. The organisms from human sources sometimes succeed in establishing themselves in udders and multiply rapidly there; it appears that they may grow without causing evident abnormalities, although usually a definite inflammation results. It

[145] See ref. 106.

[146] Jr. Med. Res. 31. P. 455. 1914.

should be noted, however, that the Streptococcus most commonly involved in udder infections is a type that is non-pathogenic for man, and that it is only an occasional udder infection that proves harmful.

The extent of some of the epidemics suggests that there must have been a heavy infection of the milk and this is much more likely when the organisms are actively developing in the udder than when they come from an infected human throat. The growth of organisms is, of course, another possibility but would not be expected with these organisms more than with other pathogenic types.

Sources of causative organisms in milk-borne septic sore throat. The sources of the causative organisms in epidemics of milk-borne septic sore throat are shown by the following summary prepared by Armstrong and Parran[147] in their tabulation of 42 epidemics:

Sources from which Milk was Infected with Organisms Causing Septic Sore Throat

Probable Source of Infection	Number of Epidemics
Cases on farms	16
Mastitis in cows	3
Cases on farms and mastitis in cows	9
Carriers on farms	2
Undetermined	12

These results emphasize the importance of both cases and abnormal udder conditions in cows as a cause of the epidemics. The association of these two causes in nine epidemics is of special interest.

Scarlet Fever

Although scarlet fever is contracted principally through direct contact, the causative organism occasionally gets into dairy products and then, when these are consumed, may reach susceptible persons. Armstrong and Parran[148] have compiled data on 40 epidemics of scarlet fever due to dairy products that occurred in the United States from 1907 to January 1, 1927, while 25 others were previously compiled. On the basis of incomplete data the 40 outbreaks involved 3,939 cases and 20 deaths. Milk was responsible in 39 epidemics and ice cream in 1; the milk was raw in 8 instances, pasteurized in 2, while in 29 the character was not reported, although presumably it was raw.

---

[147] See ref. 92.

[148] See ref. 92.

Examples of milk-borne scarlet fever. Ex. 1. During the first 20 days of January, 1907, a scarlet fever epidemic developed in several Massachusetts cities,[149] 367 cases appearing in Boston, 152 in Cambridge, 155 in Somerville and 43 in Everett, a total of 717. Of these cases, 485 or 68 per cent occurred within a period of the six days from January 7 to 12 inclusive. An investigation showed that Dealer X delivered milk to all four cities and had supplied 80 per cent of the Boston cases investigated, 85 per cent in Cambridge, 86 per cent in Somerville and 83 per cent in Everett. Dealer X delivered milk to both a wholesale and a retail trade and the cases of scarlet fever were traced almost without exception to the retail bottled milk. This milk came to Charlestown in two separate cars, each of which was filled at its own stations, and after bottling was delivered to the sections where the cases appeared. It was found that at the X milk depot a milk taster was employed whose condition indicated that he had been affected with scarlet fever and in whose family there was a person with symptoms strongly suggestive of, but not diagnostic of, this disease. Certain employees were also at work in the depot in whose families there existed cases of scarlet fever. On one of the farms supplying the milk there had been cases of illness but these were probably not scarlet fever.

Ex. 2. An outbreak of milk-borne scarlet fever occurred in Boston [150] and suburbs during the last week of April and the first week of May, 1910, that is of interest because of the number of cases involved; during a period of 13 days a total of 842 appeared.

An investigation showed that most of the cases occurred on the route of a large milk contractor. Of the 409 cases in Boston, 286 or nearly 70 per cent were on this route, and of 155 cases in Cambridge, 126 or 81 per cent were supplied from the same source; in the other cities about this proportion of the cases used milk from the dealer. On the day the epidemic reached its height 123 cases were reported. Steps were taken soon after the milk supply appeared to be involved to make it safe by pasteurization.

The dealer in question furnished milk complying with special requirements for the Jewish Passover and a number of Jewish families that previously used other milk began to use this milk on April

---

[149] Mass. State Bd. Health. 39th An. Rpt. P. 489. 1907.

[150] Mass. State Bd. Health. 42nd An. Rpt. P. 478. 1910.

24. On April 26 a Jewish child in a family that began using the special milk on April 24 became ill with scarlet fever. Altogether there were 73 cases in Boston that used milk from this special supply and 24 of these secured milk from other dealers previous to April 24. The cases due to this special milk suggested the farms on which the milk was probably infected. The dealer secured milk from about 250 farms. Eight farms were set aside to furnish the special milk; 3 of these entered into the general supply previous to April 23 and into the special supply later and, since both supplies appeared to cause infections, it would seem that the milk was infected on one of these farms. An investigation of the farms, however, failed to reveal a source of the infection.

Importance of milk as a cause of scarlet fever. The importance of milk in spreading scarlet fever is shown by data secured in Massachusetts[151] which may be summarized as follows:

Importance of Milk as a Cause of Scarlet Fever in Massachusetts

Period	Cases of Scarlet Fever			Scarlet Fever Outbreaks Traced to Milk		
	Reported	Traced to Milk		Number	Average Number per Year	Per cent of Outbreaks of All Diseases Traced to Milk
		Number	Per cent			
1907–14	70,569	2,747	3.89	10	1.25	13.89
1915–18	25,328	140	0.55	4	1.00	14.29
1919–23	46,777	53	0.11	1	0.20	5.88
1924–26	36,052	56	0.16	1	0.33	11.11

From these results it is evident that the percentage of the scarlet fever due to milk has decreased and that at present only a comparativly few cases are caused in this way.

Longevity of scarlet fever organism in dairy products. From the limited amount of data available it appears that *Streptococcus scarlatina* dies out more quickly in dairy products than most of the pathogens found in these materials. Berry[152] noted that at ice box temperature it lived for 17 days in both milk and butter.

Sources of causative organisms in milk-borne scarlet fever. In the majority of the epidemics of scarlet fever due to dairy products the causative organism has come from cases or carriers on the farms or in the plants and, accordingly, direct human infection constitutes the great danger as far as the spread of this disease through milk is concerned. A number of epidemics have also been due to the return of improperly sterilized bottles from homes in which scarlet fever was present.

---

[151] See ref. 106.

[152] See ref. 118.

In a few instances milk-borne scarlet fever epidemics have been reported as due to lesions on the udders or teats of the producing animals. Such conclusions are largely based on the presence of the abnormal condition in the animals at the time the milk was responsible for the outbreak and the proof is by no means complete. Savage[153] has suggested that cows may be responsible for the spread of diseases that are essentially human in character by acting as carriers of the organisms. The organisms may be present as secondary invaders in lesions that are essentially bovine in nature or they may even be the cause of a local infection in the cows.

DIPHTHERIA

With diphtheria, infection is very largely limited to direct contact with human sources, either cases or carriers, but occasionally eliminated organisms encounter conditions that permit of their survival for rather extended periods. *Corynebacterium diphtheriae* appears to retain its vitality for some time in dairy products. Data on 26 epidemics spread through these materials that occurred in the United States from 1907 to January 1, 1927, have been compiled by Armstrong and Parran[154] and 18 were previously summarized. The 26 epidemics, on the basis of incomplete data, included 971 cases and 6 deaths. Milk was involved in 24 instances, ice cream in 1 and butter in 1; the milk was ordinary raw milk in 7 instances, certified in 1, pasteurized in 1 and in 15 the character of the milk was not given.

Examples of milk-borne diphtheria. Ex. 1. On April 13, after a period of comparative freedom from diphtheria, 11 cases of this disease were reported to the Board of Health of Milton, Massachusetts.[155] A case had also developed on April 12 and still another appeared on April 14. All of the cases were in families supplied by Dealer A whose route was partly in Milton and partly in Dorchester. Inquiry showed that 6 cases of diphtheria had appeared in Dorchester on April 12, 19 on April 13 and 11 on April 14 and all were supplied by Dealer A. This dealer secured his supply from six producers and on the premises of one of these, C.F.J., a case of diphtheria had appeared in a grandchild on April 11; the cooler used for the milk was washed in the house by the person who took care of the child. C.F.J. also sold milk to Dealer B who dispensed it in Hyde Park. Cases of diphtheria appeared in Hyde Park, two on April 13, four on April 14, five on April 15, one on April 16, three on April 17 and one on April 19, and all were in families supplied by Dealer B. In the three localities involved, five cases of diphtheria appeared that were not supplied with milk by A or B, but the great majority were on the routes of these dealers.

---

[153] Savage, William G., Milk and the Public Health. P. 120. 1912.

[154] See ref. 92.

[155] Mass. State Bd. Health. 39th An. Rpt. P. 497. 1907.

The later appearance of the outbreak in Hyde Park than in Milton and Dorchester was probably due to the fact that Dealer A secured milk from C.F.J. in the morning and delivered it the same day, while Dealer B obtained his supply in the evening and sold it the next day. C.F.J. came down with diphtheria after the outbreak had nearly subsided. A son of Dealer A who drank milk from the C.F.J. supply was one of the earliest victims. The partner of Dealer B developed the disease on April 18 and at about the same time another case appeared in his family.

Ex. 2. Hedrich [156] has reported an instance in which diphtheria appeared to be spread through an infection of the outsides of milk bottles. About 15 positive throat swabs were received in a city laboratory, 1 or 2 a day over a period of 10 days. Nearly all the cases were under six years of age, which suggested a milk-borne epidemic. Investigation showed that most of the involved families patronized one milk dealer who served about 1,500 customers with pasteurized milk through five wagons. The small number of sporadic cases among a large number of customers was explained as follows: Among the milk handlers there was a person whose throat contained the diphtheria organism. Part of his work was to inspect bottles for cracks and he could easily have infected his hand and then the outsides of the bottles when he lifted them by the neck. The diphtheria organism presumably got into the milk when it was poured from the bottles. The epidemic was stopped by the quarantining of the carrier.

Ex. 3. A milk-borne epidemic of diphtheria that occurred in Austin, Texas, has been reported by Graham and Golaz.[157] During

---

[156] Am. Jr. Pub. Health. 8. P. 457. 1918.

[157] J. A. M. A. 79. P. 1300. 1922.

January and February 10 cases were reported, 11 new cases developed by March 20 and 23 more by March 30. Seven of the 23 cases reported late in March were students who took their meals at the same boarding house while the others were scattered irregularly in different parts of the city. The milk used at the boarding house came from a creamery owned by B, who had two dairies, B and H, several miles apart. A large percentage of the 51 cows at Dairy B had cuts and abrasions of the teats and udders due to barbed wire fences. The milking was done by S, his wife and son. Cultures were made from the throats and noses of the milkers and from four other children in the family. The proprietor, who lived about 100 yards from the dairy proper, informed the investigators that his grandchild, living with him, had been ill with throat trouble for some time. The child had not been near the dairy nor had she handled any containers or utensils during the illness. One of the cows had three teats with ulcers which healed slowly. Cultures were taken from the teats and udder of this cow and from abrasions on the teats and udders of other cows. The throat cultures of S contained the diphtheria organism. Cultures from the grandchild were positive, as were also the cultures from the one cow. Practically every diphtheria case that developed after March 15 could be traced to this dairy; most of the patients were milk drinkers but two had used no milk and were infected by butter made from the incriminated milk. A sharp drop in the new cases followed the stopping of the use of the milk from Dairy B.

Milker S insisted that the cow involved had sores on her teats when he came to work at Dairy B on January 10 and pointed out that before January 1 the milkers at Dairy B were M, his wife and child who then went to Dairy Z. At the time of this disclosure two new cases of diphtheria were reported among the customers of Dairy Z. Cultures were accordingly taken from the milkers at Dairy Z and from abrasions on the udders and teats of the cows. These were all negative except the culture from the nose of M. An examination of M showed a perforating ulcer of the nasal septum which was covered with a membranous deposit. Repeated cultures from the ulcer were positive; the organism secured killed a 350 gram guinea pig in 36 hours, while another injected pig protected by antitoxin showed no ill effects. Even with intensive treatment the ulcer retained diphtheria organisms for many weeks.

It seems probable that M infected the cow at Dairy B when he was a milker there. The cow was dry around the first of the year and was not milked until February 1; shortly after this date cases of diphtheria appeared among the customers of the dairy. The considerable number of new cases late in March is difficult to explain. The majority of the susceptible persons using the milk at that time contracted diphtheria; 12 out of 95 at the boarding club were taken sick which is about the proportion of adults not immune.

Of the 71 cases of diphtheria reported in February, March and April, 52 used milk from Dairy B. Forty-five of the cases were reported from March 22 to April 10 and 43 of these were traced to Dairy B. The importance of nose cultures is evident from the results since the throat cultures of M were negative.

Ex. 4. A milk-borne outbreak of diphtheria occurred in Westchester Co., New York,[158] and was traced to an unpasteurized certified milk supply. A number of carriers were found in and about the farm but no definite conclusion could be reached as to the actual persons infecting the milk.

Ex. 5. Henry [159] reported a milk-borne diphtheria outbreak at Williamstown, Massachusetts, involving 32 cases, that is unique in the manner in which the milk was infected. A daughter of the owner of the farm from which the milk came was found to have a sore finger. The nail had been removed by a physician and the wound was covered by a scab beneath which there was a soft, white exudate. Cultures from the sore showed the diphtheria organism. The girl had been working in the dairy all the time, either milking or helping in other ways. It is of interest to note that the wound infection did not cause cases or carriers among 15 persons on the farm, 9 of whom were from 1 to 20 years old. Later there was a second outbreak of 14 cases and it was discovered that one of the cows had a teat infected with the diphtheria organism and also that the owner of the farm had the organism in a sore on his hand. The diagnoses were made in the State Department laboratory.

Example of butter-borne diphtheria. An epidemic of butter-borne diphtheria occurred at Lewiston, Minnesota.[160] The neighborhood had been singularly free from diphtheria and scarlet fever until April. A farmer's son, away at school, was taken ill in March with what was first thought to be measles, but when removed to a hospital it was found that he had scarlet fever; at the hospital he contracted diphtheria. He returned home on April 13 and on April 20 a brother developed diphtheria while a few days later another brother contracted scarlet fever, both diseases apparently coming from infection brought home by the first boy. During the whole period the family continued to supply the local creamery with cream contrary to the regulation of the State Board of Health.

---

[158] N. Y. State Dept. Health. 41st An. Rpt. 1. P. 14. 1920.

[159] J. A. M. A. 75. P. 1715. 1920.

[160] Minn. State Bd. of Health. 3rd Bien. Rpt. P. 203. 1911.

The family was later released from quarantine and diphtheria then spread in the neighborhood by contact. Inquiry showed a number of cases in the village. A study of the village cases indicated that no ordinary source of infection existed, nor could contact with outside families be discovered, although one case was a man who had been around the country a great deal and who might have picked up his infection anywhere. The rest of the cases were unaccounted for until it was found that every affected family had used creamery butter about the proper time to account for the case. Some families used creamery butter regularly while others had used only a pound or two. It developed that, although but a small percentage of the villagers bought creamery butter at all, every infected family except the one including the man who had been around the country a great deal had used the butter at a time to account for the infection. The bulk of the butter was shipped to Philadelphia and distributed there; it was not traced in that city.

Importance of milk as a cause of diphtheria. The percentage of the diphtheria cases that are due to infected milk is shown by the following data summarized from the results secured in Massachusetts:[161]

Importance of Milk as a Cause of Diphtheria in Massachusetts

Period	Cases of Diphtheria			Diphtheria Outbreaks Traced to Milk		
	Reported	Traced to Milk		Number	Average Number per Year	Per cent of Outbreaks of All Diseases Traced to Milk
		Number	Per cent			
1907–14	69,646	131	0.18	5	0.62	6.94
1915–18	33,807	30	0.08	1	0.25	3.57
1919–23	42,386	8	0.018	1	0.20	5.88
1924–26	15,173	25	0.16	1	0.33	11.11

---

[161] See ref. 106.

These results show that milk is of comparatively little importance in the spread of diphtheria, the maximum percentage of cases that were milk-borne being only 0.18 per cent.

Growth of diphtheria organism in milk. *C. diphtheriae* is capable of growth in milk but development does not appear to be as rapid as with some of the other pathogens occasionally present in milk. Eyre[162] secured growth in milk drawn aseptically; in sterile milk, Brown[163] noted multiplication at both 36° C. (96.8° F.) and room temperature (21.7° to 25.6° C.—71° to 78° F.).

Longevity of diphtheria organism in dairy products. The diphtheria organism appears to maintain its vitality in dairy products for considerable periods. In connection with the study of a butter-borne diphtheria outbreak, the Minnesota State Board of Health[164] found the organism still alive in butter after one month.

Sources of diphtheria organism in milk. The milk-borne diphtheria epidemics have usually been the result of the direct infection of milk from cases or carriers, although occasionally the organism appears to have come from lesions on the udders and teats of the producing animals. *C. diphtheriae* is presumably present in such lesions as a secondary invader and is the result of infection from a human source.

Isolation of diphtheria organism from milk. The isolation of *C. diphtheriae* from milk has been reported by a number of investigators but the difficulties involved make negative results in attempts along this line open to serious question.

POLIOMYELITIS

One of the diseases that has been recently added to the group that is sometimes spread through milk is poliomyelitis. There is no evidence that it is commonly spread in this way but the few epidemics reported are very suggestive.

Examples of milk-borne poliomyelitis. Ex. 1. An outbreak of poliomyelitis that was apparently milk-borne has been reported by Knapp, Godfrey and Aycock.[165] During October, 1925, four cases of poliomyelitis occurred in Cortland, New York, and although their simultaneous occurrence suggested a common source of infection, this could not be located. A second group of eight cases then appeared between December 14 and 26. All of these cases, with one possible exception, had consumed milk from Dealer G. On December 7, seven days before the onset of the first of the cases, a boy who was working on the farm where the bulk of the milk sold by G was produced, became ill. He continued at work, although he noticed that his hands were growing progressively weaker and that there was pain and tenderness in his left arm. On the morning of December 11, after milking several cows, he gave up work and was taken to his home where a diagnosis of poliomyelitis was made. Five of the patients in the second group were in families that regularly secured milk from Dealer G while, with one possible exception, the others obtained milk from this source in various ways. All of the cases except one seemed to be traceable to the delivery of night milk and the milk may have been contaminated only once.

---

[162] See ref. 110.

[163] See ref. 109.

[164] See ref. 160.

[165] J. A. M. A. 87. P. 635. 1926.

Dealer G had all of the cases of poliomyelitis, with one possible exception, among the consumers of his supply which constituted less than 4 per cent of the milk delivered in the city. The cases were widely enough distributed so that it seemed improbable their relation to the milk supply was a coincidence. The actual paralytic case on one of the farms producing the milk also suggests the spread of the disease through this product.

Ex. 2. Aycock[166] has reported an epidemic of poliomyelitis that occurred in Broadstairs, England, during October, 1926. The outbreak started and subsided suddenly, 62 of the 75 cases being reported between October 14 and 29; 31 of them were reported on October 4 and the five days following. Multiple cases occurred in private boarding schools having little communication with the town or with each other. In the five years previous to the outbreak only 2 cases of poliomyelitis had been reported in Broadstairs.

Practically all of the cases were supplied with milk from Dealer A. Although three boarding schools in which cases occurred were supplied by Dealer F, these may have secured milk indirectly from Dealer A since from September 23 to October 15 he supplied 44 quarts daily to Dealer F. Four persons, who left Broadstairs shortly before the occurrence of the epidemic, developed the disease and each of these, while in Broadstairs, had lived in a house supplied by Dealer A.

Of the total 75 cases, 50 were known to have used milk from Dealer A, while 19 others (in private boarding schools) likely received this milk. All of the 43 paralyzed cases could be associated with milk from Dealer A, 35 directly and 8 through Dealer F. Dealer

---

[166] Am. Jr. Hyg. 7. P. 791. 1927.

A supplied different grades of milk including Grade A, which came from a tuberculin tested herd, and although the data were incomplete there was a high proportion of Grade A users among the cases. This suggests that the milk was infected on the farm producing the Grade A product.

CHOLERA

A number of milk-borne epidemics of cholera have been recorded and such outbreaks are to be expected from the frequent distribution of the disease through water. In certain countries in which cholera is common the practice of boiling milk probably prevents much of the infection that would otherwise occur. *Vibrio comma* is apparently rather easily destroyed by acid developed in milk or cream.

Examples of milk-borne cholera. During the World War the British Army operating in the Near East experienced acute epidemics of cholera in two hospitals[167] between August 24 and 31, 1916. In one hospital there were 35 cases with 14 deaths, and in the other 19 cases with 10 deaths. In both instances the infection was carried by the milk supply. The milk was commonly sterilized before it was given to the patients, but it often arrived late and the sterilization was not always supervised.

INFANTILE DIARRHEA

Infantile diarrhea includes a group of intestinal disturbances in infants that are responsible for a great deal of illness and many deaths, especially among children under one year of age. The condition appears to be related to the food since cases are much more common among artificially fed infants than among those that are breast fed, although it is by no means confined to the former group. It is perhaps more logical to ascribe the cases to a lack of breast feeding than to the use of other foods since human milk would be expected to be the most satisfactory material for the feeding of infants. There does not appear to be a specific organism that is regularly responsible for infantile diarrhea and each of a number of organisms has apparently been involved in certain cases; the products of the growth of bacteria may also be a factor. Infantile diarrhea is primarily a disease of the late summer months and, since a high bacterial count in the milk is more common at this season, it has sometimes been assumed to be causally related to the condition. The great prevalence of infantile diarrhea in cities, where the milk is older at the time it is consumed than in the country, suggests the same thing. More recently, however, the relationship of factors other than the quality of the milk has been shown to be important.

---

[167] Medical Services. Diseases of the War. Vol. 1. P. 119. 1922. Edited by Com. headed by Macpherson, Sir William Grant.

Park and Holt[168] have reported results secured in the feeding of infants in tenement houses and institutions in New York City with milk of various types and of varying degrees of purity; they summarized the results obtained as follows:

Results of Feeding Infants with Various Foods

(*Foods and Results, Winter and Summer*)

Milks	Infants									
	Did Well		Did Fairly		Did Badly		Died		Total	
	W *	S †	W	S	W	S	W	S	W	S
Store milk	47	21	6	23	2	20	0	15	55	79
Condensed milk	39	22	5	20	2	14	2	14	48	70
Good bottled milk	51	37	13	23	1	29	3	9	68	98
Milk from central distributing stations	35	84	20	33	4	24	0	4	59	145
Best bottled milk	5	9	0	3	1	0	0	0	6	12
Breast feeding	7	17	1	7	0	7	1	0	9	31
Totals, excluding duplicates	156	184	41	108	8	88	6	41	211	421

* Winter. † Summer

These investigators concluded that, during cool weather, neither the mortality nor the health of the infants was appreciably affected by the kind of milk or by the bacterial content, while during the hot weather when the resistance of the children was lower, the kind of milk influenced both the morbidity and mortality, those receiving breast milk, pure bottled milk or modified milk doing the best. The effect of the bacterial contamination was very marked when the milk was taken without previous heating but only slight when heating was employed shortly before feeding, unless the contamination was very excessive. The difference between the winter and summer results seemed to be due to many factors but the investigators considered that heat was the primary one and bacteria and their products of secondary importance, except when contamination was extreme or pathogenic organisms were present. In certain instances cases of diarrhea among infants getting poor milk were improved by simply giving a good quality of milk, while in other instances they were not.

---

[168] Arch. of Ped. 20. P. 881. 1903.

The relationship of the type of feeding to the deaths among infants from epidemic diarrhea is illustrated by the following data taken from the work of Newsholme[169] in Brighton, England:

Relationship of Type of Feeding to Deaths from Epidemic Diarrhea Among Infants

	Percentage of Infants under One Year of Age Fed in Various Ways	
	1,259 Infants in 10,308 Houses Visited	121 Infants Dying of Epidemic Diarrhea
1. Suckled only	62.3	6.5
Suckled and farinaceous food	12.1	2.5
Suckled and cows' milk	1.8	1.7
Suckled and condensed milk	1.4	0.8
2. Cows' milk only	7.2	36.0
Cows' milk and farinaceous food	7.6	9.1
3. Condensed milk only	3.1	30.3
Condensed milk and farinaceous food	2.0	3.2
4. Farinaceous foods only mentioned	2.0	1.7
5. Unknown	0.5	8.2
	100.0	100.0

These results show that comparatively few deaths occurred among the breast fed infants and a large number among those artificially fed. Newsholme has pointed out, however, that the deaths from diarrhea among breast fed infants show that milk is not the only cause of the disease and that the infection in these cases must have been domestic in origin; this logically leads to the conclusion that if infection was domestic in origin with the breast fed infants, it must also have been a factor with those artificially fed.

Davis,[170] working in Boston, collected data on the extent of breast and artificial feeding of infants and compared the results on the basis of the number of deaths among the two groups. The actual deaths among infants from two weeks to one year of age, in 1911, were 2,245; if all had been breast fed there would have been 1,253 deaths and if all had been bottle fed there would have been 4,352. Of the 621 deaths from diarrhea and enteritis, 87 were among breast fed infants and 534 (or 86 per cent) were among bottle fed infants; if all the infants had been breast fed the estimated deaths would have been 493 less than actually occurred.

---

[169] Jr. Hyg. 6. P. 139. 1906.

[170] Am. Jr. Pub. Health. 2. P. 67. 1912.

In New York City, Platt[171] found that among a group of infants investigated during the summer of 1914, 2.4 times as many had diarrhea among those artificially fed as among those breast fed. Among infants exposed to flies there were 1.9 times as many cases of diarrhea as among those protected from flies and among infants in dirty houses there were 1.8 times as many cases as among infants in clean houses; with flies and dirty houses combined the factor was 2.4 and with artificial feeding and dirty houses it was 3.5. Platt suggests that artificial feeding would probably have been of greater importance if the milk supply had not been rather carefully supervised.

Price[172] has reported data showing that, in Detroit, the infant deaths in a number of districts that had previously had a high rate were greatly reduced by assigning a nurse to each district for the purpose of instructing in and demonstrating the proper care and feeding of babies. There was no change in the milk supply except that an effort was made to eliminate the use of condensed milk. The reduction in the infant deaths in this district occurred at a time when in the remainder of the city there was no reduction.

From the above results and many others the conclusion that various factors have a definite influence on infant mortality, especially that due to intestinal disturbances, seems justified. The quality of milk is undoubtedly one of these but its exact importance varies with many conditions and, in certain instances, it may be entirely overshadowed by some other factor. Attempts to decrease the incidence of infantile mortality should take into account all the possible causes and should include a milk supply of a satisfactory quality.

---

[171] Am. Jr. Pub. Health. 6. P. 143. 1916.

[172] Int. Assn. Dairy and Milk Insp. 3rd Rpt. P. 95. 1914.

SUMMARY OF THE CONTAMINATION OF DAIRY PRODUCTS WITH PATHOGENS

A consideration of the spread of diseases through dairy products shows that the responsible organisms gain entrance from a variety of sources. These may be summarized as follows:

Sources from which pathogens get into milk.

1. Animals producing the milk.
    - *a.* Interior of the udder.
    - *b.* Ulcers, etc. on teats and udder.
    - *c.* Coat of the animal, feces, etc.
2. Persons in contact with milk and its derivatives in stables, in dairy plants, on routes, etc.; include true cases, carriers and individuals associating with persons that are ill.
    - *a.* Direct infection of dairy products.
    - *b.* Infection of utensils, equipment, etc.
3. Infected water.
    - *a.* Used for washing utensils, equipment, etc.
    - *b.* Splashing into milk or cream when used for cooling, etc.
4. Air and dust of stables, dairy plants, etc.
5. Flies and miscellaneous sources.

Although there are sources other than those listed above from which pathogens may get into dairy products, they represent very unusual instances and are of little significance. The sources outlined cover a period from before the milk leaves the udder until it, or the product made from it, is consumed and, with these possibilities existing, the incidence of cases of disease due to dairy products is not at all surprising.

There is a great deal of variation in the dangers from the various sources; for example, the possibility of the addition of pathogens to milk from the air of the milk room is only slight, while there have been many epidemics due to the persons coming in contact with milk. The dangers from any one of the sources vary with the different diseases. The addition of *M. tuberculosis* to the milk from the interior of the udder has undoubtedly often occurred while *E. typhosa* probably has never been added to milk from this source.

One of the important factors involved in the relationship of the various sources to the different diseases is the methods by which the causative organism leaves the body of the responsible person or animal, while the possibility of the survival of the organism after leaving the body is also a factor. Infected water is of importance in connection with the spread of typhoid through milk because the elimination of *E. typhosa* through the excreta results in the occasional infection of water. The air of the milk room would be expected to cause the infection of milk only rarely because desiccation and light rapidly destroy the pathogens that most frequently get into it.

PREVENTING THE DISSEMINATION OF DISEASES THROUGH DAIRY PRODUCTS

The evident methods of preventing the spread of diseases through dairy products are, (1) prevention of the entrance of infectious material and (2) destruction of the infectious material that may be present.

A consideration of the sources from which infectious material may get into dairy products shows that the thorough control of all of these is a tremendous undertaking that begins with the health of the producing animals and only ends when the dairy product is used. Perhaps the biggest problem is in connection with the persons coming in contact with the dairy products since any one of them may be normal on one day and capable of adding infectious material on the next. In the case of milk the attempts that are made to control all of the sources of pathogens add greatly to the cost and such specially produced milk is not available in the great majority of cities and towns. It must also be recognized that some of the attempts to prevent the entrance of infectious material to milk have not been successful and cases of disease have resulted. Epidemics of various diseases have been spread through milk produced under very careful conditions and, in a few instances, certified milk has been involved. On the other hand careful supervision has undoubtedly prevented epidemics. This is illustrated by an instance reported by North[173] in which a suspected case of scarlet fever and later a definite case were located among the milkers on a farm producing certified milk; although cases of scarlet fever were present in the community none of them were traceable to the supply involved.

The destruction of the infectious material that may be present in milk and cream is most readily carried out by proper pasteurization, and this controls the dangers up to the time of the treatment. Of course there is the possibility of the infection of milk and cream after pasteurization and it must be recognized that such instances have occurred, but this is small compared to the possibility of infection before the milk and cream reach the pasteurization plant, and can be controlled by proper equipment and methods. The pasteurization must be efficient if the anticipated protection is to be secured; a number of epidemics have been traced to milk that was improperly heated because of some defect in the equipment or its operation. Greater exposures to heat, such as those used in sterilization, are satisfactory for milk intended for certain special purposes, but are not applicable to the general milk supply because of their effect on the flavor and appearance. Proper pasteurization provides an inexpensive method of controlling the spread of diseases through dairy products and, because of the low cost, is applicable to the general supply of these materials. An improvement in the keeping quality of milk and cream also results from pasteurization and the saving derived from this may be considerable when the products are exposed to severe conditions.

---

[173] Eng. News. 62. P. 106. 1909.

POISONING FROM DAIRY PRODUCTS

Various dairy products are included in the large group of food materials which may cause poisoning. In some instances dairy products have been considered responsible for poisoning on very meager evidence, but in other cases careful investigations have completely established their relationship to the condition.

A number of ways in which dairy products may acquire poisonous properties have been suggested; these include, (1) conditions involving the producing animal, such as (*a*) the elimination of drugs, (*b*) the elimination of substances contained in the feed and (*c*) the elimination of substances resulting from a disease in the animal, and (2) conditions developing after the production of the milk, such as (*a*) the absorption of metals and (*b*) the action of micro-organisms. Of the conditions involving the producing animal the elimination of poisonous products contained in the feed is the most important and is illustrated by milk sickness which has already been described. Of the conditions developing after the production of the milk the action of micro-organisms, either directly or through products elaborated by them, is the most important.

The observations of Burke, Woodson and Heller[174] are of interest in connection with the absorption of metals. Following deaths among swine that were attributed to buttermilk held in a galvanized iron tank, these investigators carried out studies that showed buttermilk dissolved zinc from the surfaces of galvanized vessels in proportion to the acidity and time of exposure. Under the conditions of the experiment the souring of milk in galvanized containers had no deleterious effect on growth and reproduction in the rats and swine. Small amounts of zinc lactate had no marked effect on growth and reproduction of rats and swine but a relatively large amount of zinc carbonate (1 per cent or more) appeared to retard the growth and reproduction of rats.

---

[174] Jr. Dairy Sc. 11. P. 79. 1928.

Examples of poisoning with dairy products. Ex. 1. Macaulay[175] has reported an outbreak of cheese poisoning that occurred in Dover, England. The cheese involved was of the cheddar type and came from Canada; because of a rather distinctive appearance the cases investigated could be definitely traced to it. Out of a total of 135 individuals who were known to have eaten the cheese, 126, included in 43 families, were attacked. In every case the symptoms commenced a short time after a meal at which the cheese was eaten, and no other article of food was common to all the cases; the incubation periods varied from 3½ to 11 hours. Three persons, after recovery from the initial attack and not suspecting the cheese, ate the cheese a second time and experienced another attack. The symptoms very regularly included abdominal pain while diarrhea, vomiting and prostration were common. There were no deaths.

The cheese seemed perfectly sound and wholesome. A few small holes, apparently due to gas, were noted in pieces of the cheese and were not seen in other cheese of the same batch. Feeding trials with mice gave irregular results but in some instances symptoms of poisoning were noted. A rabbit that was fed twice with large doses of the cheese remained unaffected. The one trial with a human subject yielded a definite poisoning. No organism known to be associated with food poisoning was found in the cheese or in specimens from cases, and mineral poisons could not be detected. The outbreak was considered to be almost certainly due to a bacterial toxin.

Ex. 2. A sample of home made cottage cheese, which had caused the death of three persons who had eaten it, was studied by Nevin and Mann.[176] An anærobic organism was isolated that killed mice, guinea pigs and rabbits and these species were also killed by filtrates of the cultures; the minimum fatal dose of toxin was 0.0005 cc. of the filtrate of a 72 hour culture and it killed guinea pigs in four days. By repeated injections of heated toxin an antitoxin was obtained from a rabbit which protected a guinea pig against 100 times the minimum lethal dose of toxin. The organism was identified as *Clostridium botulinum.* The inoculation of 5 cc. of the toxin into a kitten gave the typical picture of botulism.

---

[175] Lancet. 203. P. 1012. 1922.

[176] N. Y. State Dept. Health. 36th An. Rpt. 3. P. 306. 1915.

Ex. 3. An outbreak of cheese poisoning involving six cases in one family occurred in Kalamazoo, Michigan, and a portion of the responsible cheese was studied by Levin.[177] The cheese was of the cheddar type and was normal in appearance and odor. When fed to a guinea pig, two rats and two mice it produced no effect. An organism was isolated from the cheese which proved pathogenic for mice, one cubic centimeter of a pure culture in milk killing a mouse on intraperitoneal injection in 8½ hours. After passage through several mice the virulence had increased so that the organism killed a rat, and after passage through a number of rats it killed a guinea pig. Injections of the original cheese into rats and guinea pigs had no effect. Rats, rabbits, cats and dogs were also found susceptible to the organism.

Filtrates from lactose broth cultures were toxic for animals; one-tenth cubic centimeter was found to be the smallest amount that would kill a 200 to 250 gram guinea pig. The toxic constituent was not destroyed by heating at boiling temperatures for one hour. The identity of the toxic agent remained undetermined. The organism was related to *E. coli.* Immunity to the organism could be established in guinea pigs by vaccine or immune serum injections.

---

[177] Jr. Lab. and Clin. Med. 2. P. 761. 1917.

CHAPTER VIII

PRESERVATION OF MILK AND CREAM

Milk and cream, even when produced under the most careful conditions, always contain micro-organisms that are ready to grow and produce changes. The suitability of these materials for the growth of micro-organisms, especially certain of the bacteria, results in very rapid transformations at favorable temperatures and makes their preservation an important problem. Although the normal milk enzymes may effect changes in milk and cream, they are of no significance in the ordinary preservation, because of their slow action, and the only factors that need to be considered are micro-organisms and the enzymes elaborated by them.

The prevention of the action of micro-organisms in milk and cream includes the following possibilities:

1. Removal of contained micro-organisms.
2. Prevention of the growth of micro-organisms.
3. Destruction of micro-organisms.

These possibilities will be considered separately.

REMOVAL OF CONTAINED MICRO-ORGANISMS

The removal of contained micro-organisms is of little practical importance in milk and cream preservation but various processes have been developed which remove, or are sometimes supposed to remove, a part of the micro-organisms and some of these are of considerable importance. Micro-organisms can not be removed from milk or cream by any system of bacteria proof filters because many of the fat globules are larger than most of the micro-organisms and would, accordingly, also be retained. The processes that require consideration are, (1) straining or filtering, (2) separation and (3) clarification.

Straining or Filtering

The straining or filtering of milk is practiced on many producing farms and also in milk plants. Various types of cloth, some of which are prepared primarily for milk filtration, are the materials most commonly used but absorbent cotton between layers of gauze is also employed. In order to prevent undue pressure at any point and to make the process easily carried out, the filtering agent is often supported by a wire strainer or some other device. Special milk filters of a number of types are also available. These are intended to make filtration practical for large volumes of milk, while some have additional advantages; for example, the milk may be passed up through the apparatus so that the sediment falls away from the filter cloth and is not broken up and driven through or into it by the pressure of the milk. Either gravity or a pump may be used to carry milk through a filter.

General influence of straining or filtering. The principal accomplishment of straining or filtering milk is the removal of a considerable portion of the visible dirt and this is ordinarily regarded as of sufficient importance to justify the practice. It would be much better if all milk could be produced under such conditions that there would be no visible dirt but, even with unusually careful methods, some sediment is commonly present. Moreover, special production methods are not applicable to milk going into the ordinary supplies because of the expense involved. If sediment is regularly present in milk, it seems logical to remove as much of it as possible, instead of consuming the milk with the sediment in it. The removal of the sediment from milk has been objected to on the basis that the purchaser is given the wrong impression of the care used in the production but it seems improbable that the purchaser familiar with the milk industry is ever misled. Straining or filtering should not be used to cover up unsatisfactory methods of production but the improvement in the appearance of milk that results from the removal of a reasonable amount of sediment is an accepted practice that is comparable to methods used in making other food products more attractive in appearance.

Influence of straining or filtering on the bacterial content. In general, the straining or filtering of milk seems to have only a negligible effect on the bacterial content. The particles of material filtered out contain considerable numbers of organisms although some of those originally present are distributed through the milk. It seems probable that the pouring or pumping of milk against a filter tends to break up some of the sediment and thus set free more organisms than if the particles of dirt had remained intact; this may be partly responsible for the slight increases in the bacterial counts sometimes noted with straining or filtering. Stocking[1] investigated the influence of straining milk on the dirt content, the bacterial content and the keeping properties. Immediately after the milk was drawn it was run through two thicknesses of fine cheese cloth supported by a wire gauze. In seven trials the dirt in the unstrained milk averaged 0.0593 grams per liter and in the strained milk 0.0317 grams; the dirt removed by straining varied from 10.8 to 91.8 per cent and averaged 46.6 per cent. The following summary shows the average numbers of bacteria, as determined by the plate method, in the unstrained and strained milk:

INFLUENCE OF STRAINING ON THE BACTERIAL CONTENT OF MILK

	Bacteria per CC.		
	Total	Acid Producers	Liquefiers
Unstrained milk	11,400	2,900	873
Strained milk	10,150	2,400	882

The total counts were higher in the strained milk in one comparison and in the unstrained in five, with one comparison showing no difference; the acid species were higher in the strained milk in three comparisons and in the unstrained in three, with one comparison showing no difference, while the liquefiers were higher in the strained milk in one comparison and in the unstrained in five, one comparison again showing no difference. In general, the counts on the milk before and after straining were so nearly the same that the differences can not be considered of significance. The length of time required to curdle at 21.1° C. (70° F.) was the same with the strained and unstrained milk in four comparisons while in the other three the strained samples curdled more quickly.

Conn and Stocking[2] made ten comparisons of the bacteria in unstrained and strained milk, using litmus lactose gelatin plates; the milk was drawn from individual cows in an ordinary pail and, after the removal of a sample, was strained through two layers of sterilized cheese cloth. The average counts are as follows:

---

[1] Conn. (Storrs) Agr. Expt. Sta. 14th An. Rpt. P. 105. 1901.

[2] Conn. (Storrs) Agr. Expt. Sta. 15th An. Rpt. P. 33. 1902–03.

INFLUENCE OF STRAINING ON THE BACTERIAL CONTENT OF MILK

	Bacteria per CC.		
	Total	Acid Producers	Liquefiers
Unstrained milk	13,743	3,523	679
Strained milk	13,005	2,703	617

The total counts were higher in the strained milk in four comparisons and in the unstrained in six, the acid species were higher in the strained milk in four comparisons and in the unstrained in six, while the liquefiers were higher in the strained milk in six comparisons and in the unstrained in four; in practically all cases, however, the differences were negligible. The time of curdling at 21.1° C. (70° F.) varied greatly but on the average the unstrained samples curdled four hours earlier than the strained.

The effect of filtering milk through three separate layers of absorbent cotton in a special apparatus used on a farm producing milk of a high sanitary quality was studied by Heinemann, Luckhardt and Hicks.[3] Samples were taken from the milk in the can, then from the top of the strainer and, finally, after straining. Five sets of samples were secured each day for 16 days. The bacterial counts were made by the plate method, using 1 per cent glucose agar. The average counts are as follows: before straining 2,060 per cc.; on top of strainer 3,912; after straining 2,790. These investigators suggest that the higher counts on top of the strainer and after straining may be due to a breaking up of the clumps of bacteria through the agitation.

Harding, Ruehle, Wilson and Smith,[4] in a study of the effect of certain dairy operations on the numbers of bacteria in milk, determined the bacterial content of the milk of one cow at various stages in its handling, including before and after straining. The utensils used were thoroughly steamed. In 17 trials the average bacterial content of the milk in the can after cooling, but before straining, was 443 per cc. while after straining it was 474; in 7 of the 17 trials a lower count was obtained after straining than before, but usually

[3] Jr. Inf. Dis. 7. P. 47. 1910.

[4] N. Y. (Geneva) Agr. Expt. Sta. Bul. 365. 1913.

the difference between the strained and unstrained samples was so small that it was easily accounted for by experimental error.

The effect of filtering milk in an apparatus in which the flow was upward through a filter cloth was studied by Lucas, Cooledge, Goodwin and Werdon.[5] The bacterial counts were determined by plating on a milk powder agar medium and incubating at 37° C. (98.6° F.) for 48 hours. With 68 lots of milk filtered at 29.4° to 32.2° C. (85° to 90° F.) the effect on the bacterial counts was as follows: 18 (26%) of the lots showed increases of from 3 to 81 per cent (av. 24.4%), 44 (65%) showed decreases of from 2 to 53 per cent (av. 19.3%), while 6 (9%) showed no change. With 74 lots filtered at 12.8° to 21.1° C. (55° to 70° F.) the following changes occurred: 20 (27%) of the lots showed increases of from 4 to 114% (av. 24.7%), 40 (54%) showed decreases of from 5 to 88% (av. 29.3%), while 14 (19%) showed no change. The results indicate that the filter tended to lower rather than increase the plate count but a reduction was by no means regularly obtained. The filter had but very little selective action in removing bacteria, although the peptonizers seemed to pass through more readily than members of the other groups. Filtration had no appreciable effect on the keeping qualities or the cream line of milk. It was very effective in removing visible dirt, being almost as efficient as clarification.

Contamination from strainers and filters. In considering the effect of straining or filtering on the bacterial content of milk, the possibility of the apparatus, especially a previously used filter cloth, being the source of large numbers of bacteria must be recognized. When a cloth is carelessly washed, without any attempt being made to sterilize it, or when it is left in a moist condition for a considerable period so that the growth of organisms can occur, a heavy contamination of the milk at the next period of use is to be expected. In general, the types of organisms added are very undesirable because of their ability to grow rapidly in milk.

A filter cloth that is to be used again should be thoroughly washed, then boiled or steamed, and quickly dried if it is to be kept in a suitable condition. This sort of treatment tends to reduce the efficiency. Lucas, Cooledge, Goodwin and Werdon[6] found that a filter cloth used once and sterilized was still fairly efficient but, for dirty milk, the efficiency was reduced to approximately 80 per cent by a second washing; they noted that, with fairly clean milk, a cloth may be successfully used longer than with dirty milk. In many milk plants employing filters, a cloth is used only once.

---

[5] Mich. Agr. Expt. Sta. Tech. Bul. 84. 1927.

[6] See ref. 5.

In two milk plants, one using a gravity filter and the other a pressure filter, Dahlberg and Marquardt[7] found that the accumulation of sediment on the filter cloth did not affect the efficiency of the filter; they pointed out that this suggests the sediment remained on the cloth and was not washed into the milk.

SEPARATION

The effect of the separation of milk on the organism present is of importance from a number of angles, such as the comparative bacterial standards for milk and cream and the bacteriology of products made from cream.

Distribution of organisms during separation. The slime which collects in the wall of a separator contains large numbers of bacteria, along with body cells, dirt and various other materials, but the amount of slime compared to the amount of milk is so small that no great elimination of organisms is to be expected. Very often the cream contains somewhat larger numbers of bacteria per cubic centimeter than the whole milk from which it came while the skim milk contains smaller numbers. The tendency to concentrate the organisms in the cream is due to the fact that they become entangled in the fat globule clusters and are thus carried into the fat rich portion. The breaking up of bacterial clumps, which has been suggested as a factor in the higher counts sometimes obtained following straining, would also be expected when the separation is carried out by centrifuging.

Eckles and Barnes[8] made a series of comparisons of the numbers of bacteria in whole milk, skim milk, cream and separator slime, using the plate method; typical results are as follows:

INFLUENCE OF SEPARATION ON THE BACTERIA IN MILK

	Bacteria per CC.		
	Trial I	Trial II	Trial III
Whole milk	58,050	207,200	206,350,480
Skim milk	16,200	81,375	67,190,000
Cream	98,000	255,780	215,488,000
Slime	37,595,250	33,226,929	3,964,713,800

---

[7] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 104. 1924.

[8] Ia. Agr. Expt. Sta. Bul. 59. 1901.

The cream contained more bacteria per cubic centimeter than the whole milk while the skim milk contained less; the bacterial content of the slime was very high.

In the data reported by Anderson,[9] the average counts obtained in seven trials, using centrifugal raising of the cream, were whole milk 619,800 per cc., cream 4,500,000 and skim milk 119,700, while in six trials with gravity separation the whole milk averaged 2,708,000 per cc. the cream 15,416,000 and the skim milk 2,050,000.

Lamson [10] noted that there were usually less or only slightly more bacteria per cubic centimeter in separator cream than in the whole milk from which it came, while the skim milk usually contained fewer bacteria than the whole milk.

Leete [11] concluded that centrifugal separation with a clean separator will not result in cream having a greatly higher count than the whole milk from which it is obtained, while gravity cream will give a much higher count than the whole milk. In 100 runs with a steam turbine separator the average bacterial content of the whole milk was 435,240 per cc., that of the skim milk 312,740 and that of the cream 500,830. Seventy samples of the cream showed increases in the count per cubic centimeter over the corresponding whole milk with an average of 23.87 per cent, 23 samples showed decreases with an average of 14.06 per cent, while with 7 samples the counts on the cream and whole milk were the same; considering the 100 samples, the average increase of the cream over the whole milk was 14.84 per cent. The fat content of the whole milk averaged 3.905 per cent while that of the cream averaged 34.64 per cent. Both the greatest increases and decreases in the bacterial content of the cream over the whole milk occurred with low count milk. The gravity separation was carried out in a can 18 inches high and 8½ inches in diameter, which was carefully washed and steamed before use. During the separation the can was kept in a tank of ice and water. In 25 runs the average bacterial content of the whole milk was 135,880 per cc., that of the skim milk 33,556 and that of the cream 283,680. The bacterial content of the cream was higher than that of the corresponding whole milk in every case, the average increase being 160.86 per cent, while the skim milk was lower in bacteria than the whole milk in 23 of the comparisons. The average fat content of the whole milk was 4.208 per cent while that of the cream was 20.52 per cent.

---

[9] U. S. Pub. Health and Mar.-Hosp. Serv. Hyg. Lab. Bul. 56. P. 739. 1909.

[10] Jr. Dairy Sc. 1. P. 498. 1918.

[11] Jr. Agr. Res. 31. P. 695. 1925.

Distribution of bacteria in bottled milk. The distribution of bacteria in bottled milk has been studied by Torrey and Rahe,[12] who found that the upper two ounces of the cream of fresh bottled milk of fair quality contained, on the average, 50 to 100 per cent more bacteria than an equal amount of the lower cream, while with older and more grossly contaminated milk the lower cream might contain as many or even more bacteria than the upper layers; a higher percentage of bacteria was brought to the surface layers in a milk rich in fat than in one poor in this constituent. After holding one hour in bottles there was no distinct cream layer and only a slight increase in bacteria at the surface and it was not until a cream layer began to form that an excess of bacteria in the upper layers was noted.

Influence of separation on pathogens. Separation does not appear to have a selective action on the organisms in milk and there is no complete concentration of the pathogens in either the slime or cream. Moore[13] studied the effect of centrifugal separation on *M. tuberculosis* in milk and found that, although many of the organisms were thrown into the slime, both the skim milk and cream also contained them.

CLARIFICATION

The clarification of milk appears to have had its beginning in the employment of a separator to throw out the foreign material gaining entrance during the production and handling. There were certain objectionable features in the use of a separator for this purpose, such as the necessity of mixing the skim milk and cream and an occasional interference with the creaming ability of the remixed milk and, accordingly, machines were designed in which the primary effect was the removal of sediment instead of the separation of the fat. Clarifiers are now widely employed in the market milk industry and their use is being advocated for milk intended for manufacturing purposes.

Composition of clarifier slime. The slime collecting in the bowl of a clarifier is made up of dirt of various kinds that has fallen into the milk, body cells, bacteria, particles of casein, fat and various other materials. Erythrocytes are present occasionally, even when the appearance of the milk does not suggest that it contains blood, and these may be deposited as a very definite layer in the slime.

The general chemical composition of clarifier slime is shown by the following data adapted from eight analyses reported by McInerney:[14]

---

[12] Jr. Inf. Dis. 7. P. 377. 1910.

[13] U. S. D. A. Yearbook. P. 431. 1895.

CHEMICAL COMPOSITION OF CLARIFIER SLIME

	Per cent H_2O	Per cent Total Solids	Per cent Fat	Per cent Ash	Per cent N	Per cent Casein
Average....	71.33	28.67	4.0	3.15	0.33	2.13
Maximum..	75.50	30.08	5.0	4.17	0.71	4.52
Minimum..	69.92	24.50	3.2	2.59	0.10	0.63

The bacterial content of clarifier slime varies a great deal, due presumably to variations in the ratio of the bacteria to the other slime constituents. Hammer,[15] working with a De Laval clarifier, noted that 36 samples of slime contained from 103,500,000 to 20,000,000,000 bacteria per gram. Hammer and Hauser[16] studied 14 samples of slime from a Sharples clarifier and found from 7,800,000 to 5,210,000,000 bacteria per gram; in general, slime with a high bacterial count came from milk containing many organisms. Results reported by Marshall and Hood[17] also indicate that the bacterial content of the milk influences the bacterial content of the slime; the numbers of bacteria in 35 samples of slime from certified milk, most of which represented milk from individual animals, ranged from 17,000 to 650,000 per gram, while the numbers in 15 samples of slime from ordinary market milk varied from 900,000 to 750,000,000 per gram.

The numbers of body cells present in clarifier slime also show a great variation. Hammer[18] found from 565,000,000 to 1,295,000,000 cells per gram in 36 samples of slime from a De Laval clarifier, while Hammer and Hauser[19] noted from 57,000,000 to 658,000,000 per gram with 14 samples from a Sharples clarifier. With 23 samples of slime from certified milk, most of which represented milk from individual animals, Marshall and Hood[20] found the cell content varying from 3,000,000 to 670,000,000 per gram, while with 6 samples from ordinary market milk the number ranged from 200,000,000 to 420,000,000 per gram.

---

[14] N. Y. (Cornell) Agr. Expt. Sta. Bul. 389. 1917.

[15] Ia. Agr. Expt. Sta. Res. Bul. 28. 1916.

[16] Ia. Agr. Expt. Sta. Res. Bul. 47. 1918.

[17] Mass. Agr. Expt. Sta. Bul. 187. 1918.

[18] See ref. 15.

[19] See ref. 16.

[20] See ref. 17.

Amount of clarifier slime. The amount of clarifier slime secured from milk is extremely variable and depends, to a large extent, on the amount of dirt and the numbers of body cells present in the milk. Other factors are undoubtedly sometimes involved; for example, the development of a small amount of acid in milk may so influence the casein that considerable quantities are thrown into the slime. In all cases, however, the amount of slime is small compared to the amount of milk clarified. McInerney[21] found in eight trials, each involving several thousand pounds of milk, that the weight of the slime varied from 0.0063 to 0.0149 per cent of the weight of the milk.

Marshall and Hood[22] reported the work of Davies on the clarification of small lots of certified milk from individual cows. The per cent slime secured in 440 trials on the milk from 74 animals averaged 0.067 per cent. It was found that different individuals varied greatly in the amount of slime given under apparently normal conditions and also that individuals often varied from one milking to the next. The amount of slime was increased by abnormalities of the udder or teats. Cows that had just freshened gave a high per cent of slime which often continued for several weeks.

Influence of clarification on the bacterial content of milk. The bacterial content of clarifier slime shows that organisms are regularly removed from milk by clarification. However, the number of bacteria, as determined by the plate method, is very often greater in clarified milk than in unclarified milk from the same lot. This increase is due to the breaking up of the clumps of bacteria during the agitation in the machine, so that a clump, which would have given only one colony before clarification, gives several colonies on the plates poured from the clarified milk; accordingly, the increase is spoken of as an apparent increase. The removal of bacteria and the breaking up of the clumps are the two factors tending to change the number of bacteria during clarification and whether there will be a decrease or an apparent increase is determined by which of these factors has the greater influence.

Hammer,[23] using the plate method, compared the bacterial content of milk before and after clarification in a De Laval machine. In 51 comparisons on milk containing less than 100,000 bacteria per cc.

[21] See ref. 14.

[22] See ref. 17.

[23] See ref. 15.

originally, 3 (6%) gave the same count before and after clarification, 14 (27%) showed a decrease of from 2 to 24 per cent (av. 12%) after clarification, while 34 (67%) gave an increase of from 2 to 256 per cent (av. 41%). In 27 comparisons on milk having between 100,000 and 500,000 bacteria per cc. originally, 9 (33%) showed a decrease of from 2 to 36 per cent (av. 12%) after clarification and 18 (67%) showed an increase of from 3 to 187 per cent (av. 43%). In 14 comparisons on milk having over 500,000 bacteria per cc. originally, 3 (21%) showed a decrease of from 5 to 40 per cent (av. 24%) after clarification and 11 (79%) showed an increase of from 4 to 102 per cent (av. 29%).

Hammer and Hauser[24] studied the effect of clarification in a Sharples clarifier on the bacterial content of milk, as determined by the plate method. Of 52 lots of milk containing less than 100,000 bacteria per cc. originally, 1 (2%) gave the same count before and after clarification, 8 (15%) showed a decrease of from 2 to 43 per cent (av. 16%) after clarification, while 43 (83%) showed an increase of from 1 to 767 per cent (av. 85%). For the 52 comparisons there was an average increase in the bacterial content of 68 per cent. In 9 comparisons on milk containing from 100,000 to 500,000 bacteria per cc. originally, 7 (78%) showed a decrease of from 9 to 55 per cent (av. 19%) after clarification, while 2 (22%) showed an increase of from 27 to 44 per cent (av. 35%). Considering the 9 comparisons there was an average decrease in the bacterial content of 7 per cent. In 32 comparisons on milk containing over 500,000 bacteria per cc. originally, 17 (53%) showed a decrease of from 1 to 31 per cent (av. 14%) after clarification, while 15 (47%) gave an increase of from 3 to 50 per cent (av. 13%). If the 32 comparisons are considered there was an average decrease in the bacterial content of 1 per cent.

McInerney,[25] using the plate method, found with 28 samples of fresh milk that clarification caused no change in 1 lot, in 1 it caused a decrease, while in 26 it caused an increase of from 1.76 to 415.10 per cent (av. 87.15%); with 17 samples of old, dirty milk clarification increased the count in all cases, the increases varying from 5.82 to 1574.70 per cent (av. 114.77%).

With 24 samples of milk low in bacteria, Sherman[26] found that clarification caused an increase in the plate counts in all cases; the

[24] See ref. 16.

[25] See ref. 14.

[26] Jr. Dairy Sc. 1. P. 272. 1917.

average counts were 4,720 per cc. before clarification and 7,120 after. Marshall and Hood [27] got both increases and decreases in the bacterial counts as a result of clarifying 37 lots of certified milk, largely from individual animals; the maximum increase was 614 per cent and the maximum decrease was 75 per cent.

Lucas, Cooledge, Goodwin and Werdon [28] studied the effect of clarification on the bacterial content of 49 samples of milk, using temperatures ranging from 29.4° to 33.3° C. (85° to 92° F.); after clarification, 35 (71%) showed increases which averaged 28.1 per cent (from 1 to 100%), 9 (19%) showed decreases which averaged 11.7 per cent (from 2 to 23%), while 5 (10%) showed no change. Clarification at 12.8° to 18.3° C. (55° to 65° F.) influenced the bacterial content of 97 samples as follows: with 60 (62%) there were increases which averaged 25.9 per cent (from 2 to 220%), with 23 (24%) there were decreases which averaged 12.2 per cent (from 3 to 26%), while with 14 (14%) there was no change.

The effect of clarification on the bacterial content of milk, when the flow through the machine was greatly reduced, was studied by Wilster [29] at the Iowa Agricultural Experiment Station. In all cases a reduction in the count was noted.

Influence of clarification on the body cell content of milk. Because the body cells in milk are counted directly and there is no opportunity for apparent changes in number, the presence of body cells in clarifier slime indicates that clarified milk has a lower cell count than the same milk before clarification. Hammer [30] found that 52 lots of milk clarified with a De Laval clarifier showed decreases in the cell content of from 7 to 73 per cent (av. 39%). In 63 lots clarified with a Sharples machine, Hammer and Hauser [31] observed decreases of from 11 to 78 per cent (av. 41%). In neither series of trials was there any apparent relationship between the percentage of cells removed and such factors as the original cell content, the per cent of fat in the milk or the temperature of clarification. Marshall and Hood [32] found, with 25 samples of certified milk coming largely from individual animals, that clarification reduced the cell content from 46 to 92 per cent, while with 6 samples of ordinary market milk the cell content was reduced from 74 to 92 per cent.

---

[27] See ref. 17.

[28] See ref. 5.

[29] Thesis Iowa State College. The Clarification of Milk for American Cheddar Cheese. 1928.

[30] See ref. 15.

[31] See ref. 16.

[32] See ref. 17.

Removal of visible dirt by clarification. Clarification very efficiently removes the visible dirt from milk. McInerney[33] found about 99 per cent of the dirt taken out, while Lucas, Cooledge, Goodwin and Werdon[34] found over 99 per cent removed. It should be noted that a portion of the dirt which gets into milk either goes into solution or else is so finely divided it is not ordinarily visible and, accordingly, a considerable percentage of the dirt which gains entrance to milk may not be removed by clarification. Different types of dirt vary a great deal in this respect.

Effect of clarification on the keeping quality of milk. Clarification has little if any effect on the keeping quality of milk. Hammer and Hauser,[35] using room temperature, compared the acid development of 26 samples of milk before and after clarification. The titrations were made following a certain amount of curdling, while in a few instances coagulation was complete. In 3 comparisons (12%) the acidity was the same, in 13 (50%) the clarified milk showed the higher acidity, while in 10 (38%) the unclarified showed the higher acidity. These investigators concluded that clarification can not be considered to improve the keeping quality of milk under ordinary conditions. McInerney[36] compared the development of bacteria in milk before and after clarification by holding samples at 14.4° C. (57.9° F.) for 24 hours and then plating on lactose agar and incubating 3 days at 37° C. (98.6° F.). With clean milk the unclarified samples showed a greater percentage increase than the clarified in 9 of the 15 comparisons. The average increase for the 15 trials was 1,374.50 per cent with the unclarified milk and 754.29 per cent with the clarified. With dirty milk the unclarified samples showed a greater percentage increase than the clarified in 9 of 20 comparisons; the average increase for the unclarified milk was 1,691.86 per cent and for the clarified 431.50 per cent. McInerney also made 17 comparisons of the acidity of unclarified and clarified milk after holding 24 hours at 21.1° C. (70° F.) and found practically no difference, the average acidity for the unclarified milk being 0.57 per cent and for the clarified milk 0.60 per cent.

In trials with milk having a low bacterial count, Sherman[37] found that clarified milk reduced methylene blue and developed acid somewhat more rapidly than the unclarified. Lucas, Cooledge, Goodwin and Werdon[38] also concluded that clarification slightly reduces the keeping quality of milk and attributed this to a stimulation of the acid producing bacteria.

---

[33] See ref. 14.

[34] See ref. 5.

[35] See ref. 16.

[36] See ref. 14.

[37] See ref. 26.

[38] See ref. 5.

Effect of clarification on pathogens. There is no evidence to indicate that clarification makes milk safe through the removal of pathogens. Traum and Hart [39] inoculated 14 samples of clarified milk from a tuberculous herd into guinea pigs; 11 samples caused tuberculosis in all of the animals inoculated, 2 produced tuberculosis in one guinea pig while the other pig died soon after inoculation and 1 caused the death of both guinea pigs through septicemia.

Value of clarification. The value of clarification lies entirely in the removal of dirt from milk. While it would be much better if the dirt could be kept out, this is impossible with our present methods of production, except at a considerable increase in cost and then only in part. The next best procedure is to permit the removal of the visible dirt, when it is not excessive, and follow this by efficient pasteurization so that the safety of the milk is assured. The elimination of a considerable percentage of the body cells is another advantage of clarification; these cells can not be considered harmful, but their removal is advisable from the æsthetic standpoint. While clarifier slime contains large numbers of bacteria per gram, the amount of slime compared to the amount of milk is so small that clarification is of no importance in reducing the bacterial content. The effect of clarification on the keeping quality of milk is also a negligible one. The results of various investigations show that clarification does not significantly influence the creaming ability of milk.

PREVENTION OF THE GROWTH OF MICRO-ORGANISMS

The growth of micro-organisms in milk and cream may be prevented by, (1) low temperatures or (2) preservatives. Low temperatures are widely used and constitute the accepted method of holding these products, while preservatives are rarely employed, except in the samples intended for analyses.

Low Temperatures

The influence of various temperatures on the growth of micro-organisms in milk and cream is discussed in Chapter V. At 0° C. (32° F.) milk can be held for from one to three weeks before growth begins but multiplication then goes on until large numbers of organisms are present. The bacteria which develop bring about changes very different than the ordinary souring of milk and, although there is an increase in acidity, the protein decomposition is the significant change. At temperatures such as 4.4° C. (40° F.), the development of bacteria is somewhat more rapid than at 0° C., but the changes produced are of the same general character. Bacterial growth can be prevented at 10° C. (50° F.) or even somewhat higher for a certain time, the length of which is largely dependent on the types of bacteria present; these types are, in turn, determined by the sources of the contamination.

---

[39] Jr. Am. Vet. Med. Assn. 49. P. 678. 1916.

Many efficient milk plants hold the refrigerators at approximately 4.4° C. (40° F.) and this prevents the multiplication of bacteria for as long a period as milk and cream are held under ordinary conditions. When these products are to be stored for several days or more, temperatures such as 0° C. (32° F.) or even slightly lower are employed. Such storage is especially common with cream intended for the manufacture of ice cream, because of the fluctuating demand for the product and the necessity of having an adequate supply. When the period of holding does not exceed 10 days there can be no criticism of this method of preservation but, when the holding period is longer, it is open to serious objections because of the possibility of the formation of toxic products as a result of protein decomposition.

PRESERVATIVES

Milk preservatives, some of which were put out under trade names, seem to have been rather widely used at one time, but regulations prohibiting this practice have very largely eliminated it. The present availability of ice and refrigerating machinery has provided adequate facilities for the proper holding of milk and cream for periods as extended as they need to be held, so there is no longer an excuse for the use of preservatives, except in samples intended for some such purpose as chemical analysis.

General effect of preservatives on the micro-organisms in milk. The action of a preservative on the micro-organisms in milk and cream depends on the amount used. A given concentration may simply prevent growth or decrease its rate, while a higher concentration may destroy organisms. The length of time a given preservative in a certain concentration will prevent the development of changes in milk varies a great deal and depends on the types of micro-organisms present and the temperature of holding. A preservative commonly acts differently on different organisms and, as a result, the proportion of the various types present in a lot of milk or cream may be definitely modified. This may be very objectionable if the acid producing organisms are prevented from growing and those attacking the protein are not so effectively checked. In general, the lower the temperature of holding milk or cream containing a preservative, the longer the period during which the organisms are restrained.

Preservatives that have been used in milk. The more important of the preservatives that have been used in milk and cream are formaldehyde, boric acid, borax, benzoic acid, salicylic acid and hydrogen peroxide. Carbonates have also been employed but their main effect is to neutralize the acid produced rather than to prevent the growth of organisms.

Formaldehyde. Formaldehyde is very effective in preventing the growth of organisms in milk and cream and its action is illustrated by the work of Chester and Brown.[40] These investigators found that when the concentration of formaldehyde in milk was from 1 to 2,000 up to 1 to 800 there was a rapid decrease in bacteria during the first 24 hours and then a slow decrease until, at the end of 5 days, the milk was practically sterile or contained only resistant spores. With a proportion of 1 to 5,000 there was a rapid reduction in the bacteria for from 4 to 6 hours and then a slower decrease, after which multiplication began. A proportion of 1 to 10,000 gave, in some cases, a slight decrease before the increase started, while 1 to 20,000 simply slowed up the increase for a time. With a proportion of 1 to 40,000 the increase in bacteria was rapid from the first but less rapid than without formaldehyde and the milk kept two to three times as long as untreated milk. *S. lactis* appeared to develop better in the presence of formaldehyde than the other bacteria ordinarily found in milk.

Boric acid, borax, benzoic acid and salicylic acid. These chemicals definitely inhibit the growth of organisms in milk and cream. Boric acid and borax appear to have been more extensively used than the other compounds.

Hydrogen peroxide. The use of hydrogen peroxide in combination with heat has been introduced as a method of treating milk by Budde and the product is called Buddeised milk. The milk is heated to 50° C. (122° F.) and enough pure hydrogen peroxide added to give a concentration of 0.03 to 0.035 per cent. After stirring for from 15 to 30 minutes the milk is drawn into bottles, tightly stoppered, the bottles held in a water bath at 50° C. for 2 to 3 hours, and then cooled. It appears that pathogens may not be destroyed by this method. An excess of hydrogen peroxide gives an off flavor to the milk.

---

[40] Del. Agr. Expt. Sta. Bul. 71. 1905.

Preservation of milk and cream for analytical purposes. Milk and cream intended for analytical purposes may be preserved at a low temperature or by the addition of some preservative that will not interfere with the analyses to be made. Samples for fat determinations must often be held for extended periods at temperatures very favorable for growth and bichloride of mercury is frequently used for their preservation because of its toxic effect on all types of micro-organisms. The chemical is available in tablets which also contain some coloring material so that the preserved milk or cream will have a very abnormal appearance and not be consumed.

DESTRUCTION OF MICRO-ORGANISMS

The important procedures by which the destruction of microorganisms in milk and cream is accomplished under practical conditions involve the application of heat. Complete destruction of the organisms is employed with evaporated milk but is not used with milk and cream for direct consumption or for most manufacturing purposes, because of the physical and chemical changes resulting. A partial destruction of the organisms is, however, extensively employed and has been a significant factor in the development of the dairy industry; when carried out under conditions which meet certain requirements, it is referred to as pasteurization.

Pasteurization. Pasteurization, as applied to milk, cream and other dairy products, is usually understood to be a process of heating to a temperature considerably below the boiling point with the object of destroying a large percentage of the micro-organisms that may be present, including all of the pathogenic types. The exposure to heat involves two factors, temperature and time, and one is just as important as the other; a temperature which is ineffective if used for a short period may be entirely satisfactory if the time is prolonged. Cooling to a reasonably low temperature immediately following the heating, in order to prevent the growth of the organisms surviving pasteurization, is commonly understood to be a part of the process.

The pasteurization exposures demanded in different regulations vary somewhat but the object, in all cases, is to insure the destruction of the pathogens that may be present by providing a reasonable margin of safety over the actual heating required. Because *M. tuberculosis* is more resistant to heat than the other pathogens found in milk, its resistance is usually used as a basis in establishing pasteurization regulations.

Origin of pasteurization. The word pasteurization is derived from the name of Louis Pasteur, a French scientist, who developed the process. Pasteur was interested in preventing abnormalities in wine, such as the formation of acid, ropiness, etc., and found (1860 to 1864) that by heating the wine for a few moments to a temperature of from 50° to 60° C. (122° to 140° F.) the undesirable changes could be prevented. In 1868, heated wine was successfully sent around the world in the French frigate La Sybile. After the Franco-Prussian war Pasteur studied the diseases of beer and found that heating prevented undesirable changes in this product also. Pasteur was not the first to use heat to control fermentations since Scheele, as early as 1782, heated vinegar to prevent spoilage and Appert, in 1810, reported a method of preserving food materials of all sorts by the application of heat to closed containers. It should be noted, however, that the studies of Scheele and Appert were carried out at a time when the nature of fermentations was not understood, while those of Pasteur followed a knowledge of the role of micro-organisms in these changes. In 1886, Soxhlet advised the heating of milk for infant feeding; the milk needed for a day was divided and that required for each feeding was put into a bottle of a type that could be easily cleaned and sterilized, after which the bottles were heated in hot water. Soxhlet, however, incorrectly believed that milk treated according to this method was sterile.

Types of pasteurization. With dairy products two main types of pasteurization have been developed, (1) the flash method and (2) the holding method.

The flash or continuous method of pasteurization consists of heating to a comparatively high temperature for a very short period, the material running through the pasteurizer and to the cooler in a continuous stream. The temperatures used ordinarily range from 71.1° to 85° C. (160° to 185° F.) or even higher, while the time usually varies from one-half to one minute, depending on the rate at which the machine is operated. With flash pasteurization the time factor need not be stated since it is practically constant and is understood from the method. Formerly flash pasteurization was widely used with market milk but at present the holding method is the one preferred by health officials and often demanded by city ordinances. With market milk the pasteurization temperature must not be too high, because of the influence on the flavor and the cream line, and with a low temperature a satisfactory destruction of organisms, in the time which the flash method permits, is difficult; moreover, the tendency to keep the temperature down sometimes results in the use of very ineffective exposures. Perhaps the main reason the flash method was selected by many milk plant operators, when pasteurization was first used in their plants, is that bottling can be begun very soon after the milk begins to arrive and this permitted finishing the day's work somewhat earlier than with the holding method. Flash pasteurization is widely used with cream intended for the manufacture of butter; the effects of high temperatures are not objectionable from the standpoint of buttermaking and this allows the use of temperatures which give a satisfactory destruction of organisms.

The holding or discontinuous method of pasteurization consists of heating to a comparatively low temperature for a considerable period. The temperature and time of heating constitute the pasteurization exposure and both must be stated when this method of pasteurization is considered. The usual exposures are from 60° to 64.4° C. (140° to 148° F.) for from 20 to 30 minutes although, with cream intended for buttermaking, exposures approximating 85° C. (185° F.) for 20 minutes have sometimes been employed. Large vats have proved unsatisfactory for the pasteurization of market milk, because of the time required for heating and cooling, and a heating unit, from which the milk runs to a series of small holding compartments that are filled and emptied automatically or to a long system of tubes which maintains the desired temperature for the proper time, has come into use in the larger plants. The pasteurization of milk and cream in bottles, which is referred to as final package pasteurization, is essentially a holding method; the chief advantage lies in the fact that contamination after pasteurization is largely eliminated.

The basis for the present exposures used in pasteurization. Three effects of the heat applied to milk must be taken into account whenever a pasteurization exposure is under consideration. These are, (1) the bacterial efficiency, (2) the influence on the flavor and (3) the influence on the creaming ability. The first two of these apply to cream and such materials as ice cream mixes in addition to milk, while the third applies primarily to milk. If the bacterial efficiency were the only consideration, high temperatures for extended periods might be advisable but the influence of the heat on the flavor and creaming ability requires that the exposures be kept as low as possible. The heating has a desirable effect, as far as the bacterial efficiency is concerned, but an objectionable one on the flavor and creaming ability and, accordingly, the lowest exposure which will give a satisfactory destruction of bacteria is the one used for the routine pasteurization of market milk and cream.

The bacterial efficiency. To be satisfactory from the standpoint of bacterial efficiency, the heating must kill a reasonable percentage of the total bacteria present, including all of the pathogens. The bacterial efficiency may be expressed as the percentage of the total bacteria that are killed during the pasteurization, but this is not entirely satisfactory because, with excessive numbers of bacteria, a very large percentage may be destroyed by exposures that still leave many organisms and do not kill all the pathogens. In general, it can be said that, with a given exposure, the higher the initial count the greater will be the percentage of organisms destroyed; this is due to the fact that high counts on raw milk usually include many *S. lactis* organisms that are rather easily killed by heat. At present, requirements dealing with the destruction of bacteria during pasteurization ordinarily refer to the numbers permitted both before and after the heating.

The destruction of the pathogens that may be in milk is the important point to consider with reference to the bacterial efficiency of a pasteurization exposure. In deciding on the necessary exposure *M. tuberculosis* is given the principal consideration because of its high resistance compared to the other pathogens in milk.

Heat resistance of M. tuberculosis in milk. The early investigations on the heat exposure required to destroy *M. tuberculosis* in milk yielded variable results,[41] with the findings of one investigator often showing pronounced variations. In 1899, Smith[42] explained this by pointing out that the formation of a scum or pellicle on the milk, due to evaporation, enabled the organism to resist exposures that otherwise would destroy it. He found that, when suspended in milk, *M. tuberculosis* was killed at 60° C. (140° F.) in 15 minutes when no pellicle was allowed to form, while with a pellicle it might survive 60° C. for 60 minutes. Russell and Hastings[43] reported that the

[41] U. S. Pub. Health and Mar-Hosp. Serv. Hyg. Lab. Bul. 56. P. 683. 1909.

[42] Jr. Expt. Med. 4. P. 217. 1899.

[43] Wis. Agr. Expt. Sta. 17th An. Rpt. P. 147. 1900.

heating of tuberculous milk in a tightly closed commercial pasteurizer to 60° C. for 10 minutes destroyed *M. tuberculosis;* they recommended an exposure of 60° C. for not less than 20 minutes in order to insure thorough pasteurization. Later these investigators[44] reported results obtained with short periods of heating and concluded that 71.1° C. (160° F.) or above for 1 minute suffices to destroy the bovine cultures so that the disease is not produced in guinea pigs inoculated with organisms in amounts ranging from 2 to 5 milligrams. Rosenau[45] found that the organism in milk was killed when heated to 60° C. for 20 minutes or to 65° C. (149° F.) for a much shorter time.

The studies of Traum and Hart[46] are particularly interesting since they involved milk from a diseased herd that was intended for consumption after pasteurization under commercial conditions. The milk came from a heavily infected herd with which an attempt was being made to build up a healthy herd. The reacting animals, which at one time numbered 500, were kept separate from the normal ones and the milk from them was sold after it had been pasteurized. The pasteurization was carried out in three well insulated vats. Steam was turned into the circulating water as soon as milk began to flow into a vat and from 20 to 30 minutes were required to fill the vat and bring the milk to the pasteurizing temperature; the emptying of the vat required 15 or 20 minutes, so that the last milk leaving got considerable additional exposure before running over the cooler. The temperatures were secured with a recording thermometer but, part of the time, checks were run with a thermometer placed directly in the milk. The samples were preserved at the beginning of the work with 0.5 per cent boric acid and later with 1 per cent; it was found that such preservation did not destroy the tubercle bacilli. The milk was examined at a distant point, within 24 to 48 hours of the collection, by centrifuging 90 to 95 cc. and inoculating the cream and sediment subcutaneously or intramuscularly into two or three guinea pigs; reinoculations were made in all cases of suspicious lesions, even when tubercle bacilli were not demonstrated microscopically. Of the 24 samples of raw milk studied, all but 1 produced tuberculosis in inoculated animals and, with it, the guinea pigs died from septicemia; with 2 samples, one animal developed tuberculosis while the other remained well and, with 3 samples, one animal developed tuberculosis while the other died soon after inocu-

[44] Wis. Agr. Expt. Sta. 21st An. Rpt. P. 178. 1904.

[45] U. S. Pub. Health and Mar.-Hosp. Serv. Hyg. Lab. Bul. 42. 1908.

[46] See ref. 39.

lation. Following pasteurization, 23 samples failed to produce tuberculosis or cause death in a single instance; 12 of the samples were heated above 60° C. (140° F.) for longer than 20 minutes while the remaining 11 were heated to 60° C. and held for only 20 minutes.

Bartlett[47] reported that pasteurization at 61.1° to 64.4° C. (142° to 148° F.) for 30 minutes under commercial conditions destroyed *M. tuberculosis* in milk. Campbell-Brown[48] studied 25 strains of *M. tuberculosis* and found no wide difference in the thermal death point; the human and bovine types seemed to be similar in their resistance to heat. This investigator concluded that at 60° C. (140° F.) it requires 20 minutes' exposure to prevent infected milk from causing the disease in guinea pigs, while at 70° C. (158° F.) it requires 5 minutes' exposure. In the studies carried out by Cameron-Macaulay,[49] *M. tuberculosis* from sputum and from tuberculous glands was destroyed by heating in milk from 62.8° to 65.6° C. (145° to 150° F.) for 30 minutes. Jenkins[50] found that the organism in naturally infected milk was killed at 60° to 62.8° C. for 30 minutes.

It should be noted that, in studies carried out since Smith recognized the importance of preventing pellicle formation on the milk being heated, exposures generally considered to destroy *M. tuberculosis* have occasionally failed to do so. White[51] injected 30 guinea pigs with naturally infected milk, after heating it to 62.5° C. (144.5° F.) for 30 minutes, and noted 1 positive result; he stated that it was possible an accident had occurred. Meanwell[52] carried out a series of tests with naturally infected milk and secured data as follows: after heating to 62.8° C. (145° F.) for 30 minutes, 136 pigs were negative and 2 positive; after heating to 60° C. (140° F.) for 30 minutes, 96 pigs were negative and 0 positive; after heating to 60° C. for 20 minutes, 50 were negative and 2 positive; after heating to 59.3° C. (138.8° F.) for 20 minutes, 3 pigs were negative and 10 positive.

In extensive trials carried out by a group of investigators[53] working with commercial equipment, the bovine tubercle bacilli used were destroyed in some of the tests in three types of improved commercial pasteurizers at 58.9° C. (138° F.) for 30 minutes. The studies indicated the necessity of having properly designed pasteurizers if the destruction of pathogenic organisms is to be accomplished under practical conditions.

---

[47] Am. Jr. Pub. Health. 13. P. 807. 1923.

[48] Lancet. 205. P. 317. 1923.

[49] Pub. Health. 38. P. 297. 1925.

[50] Jr. Hyg. 25. P. 273. 1926.

[51] Lancet. 210. P. 223. 1926.

[52] Jr. Hyg. 26. P. 392. 1927.

[53] U. S. Pub. Health Serv. Pub. Health Bul. 147. 1925.

Park[54] has reported the thermal death point of *M. tuberculosis* in milk, exposed for from 1 to 30 minutes, as follows:

Thermal Death Point of *M. Tuberculosis* in Milk

Temperature	Time Minutes
68.3° C. (155° F.)	1
62.8° C. (145° F.)	6
61.1° C. (142° F.)	10
60° C. (140° F.)	15
58.9° C. (138° F.)	20
57.8° C. (136° F.)	30

Heat resistance of pathogens, other than M. tuberculosis, in milk. The evidence available indicates that *M. tuberculosis* is considerably more resistant to heat than the other pathogens that may be present in milk. Rosenau[55] studied the heat resistance of a number of these organisms. *E. typhosa* was killed in milk heated to 60° C. (140° F.) and maintained at this temperature for 2 minutes; the great majority of the organisms were killed by the time the milk reached 59° C. (138.2° F.), while a few survived at 60° C. The diphtheria organism often failed to grow after the milk reached 55° C. (131° F.) although, occasionally, it survived until the milk reached 60° C. The resistance of the cholera organism was similar to that of *C. diphtheriae;* it was usually destroyed when the milk reached 55° C. but once it lived until 60° C. was reached. The dysentery organisms (Shiga and Flexner) withstood 60° C. for 5 minutes but were killed at this temperature held for 10 minutes. *Br. melitensis* was not destroyed at 55° C. for a short time but was killed at 60° C. and the majority of the organisms were dead at 58° C. (136.4° F.).

Rosenow and Hess[56] found that an organism responsible for an outbreak of septic sore throat was destroyed at 60° C. (140° F.) for 20 minutes.

---

[54] Am. Jr. Pub. Health. 17. P. 36. 1927.

[55] See ref. 45.

[56] J. A. M. A. 68. P. 1305. 1917.

In trials carried out with commercial pasteurizers results as follows were secured by a group of investigators:[57] the typhoid bacilli used were destroyed in one series of tests by momentary heating in a commercial heater to 60.6° C. (141° F.) and by one type of commercial holder at 55.6° C. (132° F.) for 30 minutes; the diphtheria organisms and hemolytic streptococci used were destroyed by momentary heating in a commercial heater to 53.9° C. (129° F.).

Carpenter and Boak[58] noted that human, porcine and bovine cultures of *Br. melitensis* were not viable after an exposure to 60° C. (140° F.) for 20 minutes. Guinea pig inoculations were used in testing the heated organisms and were found to be more reliable than cultures; in a few instances cultures were negative when inoculated guinea pigs became infected.

Exposures for commercial pasteurization. The exposures used for commercial pasteurization are regularly above those required for the destruction of *M. tuberculosis* in carefully controlled tests. Under plant conditions temperature and time can not be measured as accurately as in the laboratory; the temperature especially may deviate from what is intended as a result of variations in different parts of the milk, inaccuracies in thermometers, and the failure to carefully control the heating. This necessitates the inclusion of a considerable margin of safety in the pasteurization exposure if the complete destruction of the pathogens that may be present in the milk or cream is to be assured.

The pasteurization exposures required by municipal regulations are illustrated by data collected by the Bureau of Dairy Industry of the United States Department of Agriculture and given in the table on page 261.[59]

The high bacterial counts that were sometimes obtained on pasteurized milk constitute one of the reasons for including a generous margin of safety in the exposures required. In many instances these high counts were due, in part, to contamination after heating but they were also influenced by the resistance of various non-spore forming organisms that were present in the original milk. Apparently, with a 30 minute heating period, the range from 60° to 62.8° C. (140° to 145° F.) is a critical one, as far as the destruction of many organisms is concerned, so that a slight increase in temperature may considerably increase the numbers of bacteria killed.

---

[57] See ref. 53.

[58] Jr. Bact. 15. P. 41. 1928.

[59] U. S. D. A. B. D. I. Milk Inspector Letter No. 124. Feb., 1928.

MUNICIPAL PASTEURIZATION REQUIREMENTS

Temperature	Number of Cities	Maximum Time, Minutes	Minimum Time, Minutes	Average Time, Minutes
60.0° C. (140° F.)	51	30	20	28.4
61.1° C. (142° F.)	149	30	30	30.0
61.7° C. (143° F.)	9	30	30	30.0
62.2° C. (144° F.)	5	30	30	30.0
62.8° C. (145° F.)	88	30	20	28.8
64.4° C. (148° F.)	1	15	15	15.0
65.6° C. (150° F.)	3	20	15	18.3
66.7° C. (152° F.)	1	10	10	10.0
68.3° C. (155° F.)	3	15	5	10.0
69.4° C. (157° F.)	1	20	20	20.0
70.0° C. (158° F.)	1	3	3	3.0
71.1° C. (160° F.)	2	10	5	7.5
73.9° C. (165° F.)	4	1	0.5	0.6
76.7° C. (170° F.)	1	flash	flash	flash
79.4° C. (175° F.)	1	flash	flash	flash
80.0° C. (176° F.)	1	flash	flash	flash

Influence of pellicle formation on the heat resistance of organisms. It would be expected that the protection given *M. tuberculosis* by the pellicle formed on heated milk would also occur with other organisms. Russell and Hastings[60] inoculated milk with a coccus and found that the pellicle which developed on heating the milk gave growth when the milk below did not. The removal of a pellicle resulted in the development of another, but this was sterile. When a pellicle containing organisms was heated in water, the organisms resisted temperatures that ordinarily killed them. No appreciable diminution in temperature could be detected at the surface of heated milk on which a pellicle was forming.

It appears that the increased resistance of organisms in the pellicle developed on heated milk is due to more than one factor. The pellicle forms as a result of evaporation and this requires heat so that the temperature is undoubtedly lowered, even if no decrease can be observed by ordinary measurements. It is probable also that the pellicle offers some protection, either by its resistance to the penetration of heat or in some other way. The influence of pellicle formation on the survival of organisms during pasteurization is of importance under practical conditions and pellicle development should be prevented, either by agitating the material being heated or by closing the covers of holding compartments to limit evaporation.

---

[60] Wis. Agr. Expt. Sta. 18th An. Rpt. P. 185. 1901.

Importance of uniform heating during pasteurization. A reasonable uniformity in both the temperature and time of heating the different portions of a batch of milk or cream is highly desirable. It is essential that all the milk or cream be heated sufficiently to accomplish the destruction of the pathogens, while the over heating of portions may give a pronounced heated flavor and, in the case of milk, a serious decrease in the creaming ability.

The uniform heating of a large volume of milk or cream presents a difficult engineering problem and many types of pasteurization equipment have defects of one sort or another. Some of the common causes of under heating a portion of a batch of milk or cream are (1) leaky valves which permit material to leave the equipment before the exposure is complete, (2) dead ends which retain material in such a way that it does not properly mix with that coming in contact with the heating surface, (3) splashing which carries material to parts of the equipment where it does not receive sufficient heating, and (4) the formation of foam through which the heat is transferred only slowly. Over heating may result from (1) a high temperature heating surface which supplies heat so rapidly that the material next to it is heated above the temperature desired for the entire batch and (2) from unnecessarily long holding, such as occurs when the equipment heats or cools so slowly that the material is at temperatures approximating the pasteurization temperature for extended periods.

The formation of foam on milk or cream that is being pasteurized is of considerable bacteriological significance. Whittaker, Archibald, Leete and Miller [61] studied the temperatures of milk and foam during commercial pasteurization by means of thermocouples and found, in 19 runs, that the temperature of the foam was always lower than that of the corresponding milk. At the beginning of the holding period the average temperature of the foam was 2.8° C. (5° F.) or more below that of the milk in 84.2 per cent of the tests, while the same thing was true in 73.3 per cent of the tests at the middle of the holding period and in 84.6 per cent of the tests at the end of the holding period; the temperature of the foam was frequently more than 6.1° C. (11° F.) below that of the milk and in 1 instance was 10.1° C. (18.1° F.) below it. The foam and the corresponding milk were also examined for numbers of bacteria and, at both the beginning and end of the holding, the foam showed the higher count in every instance. While the milk gave the expected decrease in the number of bacteria during holding, 66.7 per cent of the foam samples gave a higher count after holding than before; the average increase in these samples during holding was 176.3 per cent. There was a reduction in the bacterial content of the foam in vats when the covers were tightly closed, while in vats with the covers open or loose fitting there was an increase; in agreement with this, the average temperature of the foam increased approximately 1.7° C. (3° F.) during the holding in vats with the covers tightly closed, while there was no increase when the covers were raised. At both the beginning and end of the holding period there were wide variations in the bacterial content of samples of foam taken at different places in a vat. The butterfat content of foam varied from that of the corresponding milk and was higher in nearly all cases; at the end of the holding period the per cent fat in the foam was usually higher than at the beginning.

---

[61] U. S. D. A. Tech. Bul. 18. 1927.

Cooling after pasteurization. The necessity for cooling to a low temperature immediately after pasteurization is now generally recognized and is demanded by many of the regulations governing pasteurization. Cooling to temperatures that will inhibit bacterial growth is not necessary when the product is to be used in some process requiring the growth of bacteria, such as the ripening of cream for buttermaking or the preparation of certain cheeses, but it is necessary with market milk and cream, ice cream mixes, etc., in which the growth of bacteria is always objectionable and likely to result in the development of various abnormal conditions. Pasteurization, when properly carried out, destroys a large percentage of the bacteria present but can not prevent the growth of those remaining, or of those that gain entrance during the subsequent handling. The activity of such organisms must be prevented by low temperatures and the sooner these temperatures are reached, the better from the standpoint of the control of bacterial growth.

The sudden chilling of the milk, after it has been at a pasteurizing temperature, has sometimes been considered to have a destructive action on the remaining organisms. Ayers and Johnson [62] pasteurized milk in sterile flasks and then cooled it rapidly by running it through a sterile copper coil surrounded by brine; the bacterial counts before and after cooling were as follows:

---

[62] U. S. D. A. B. A. I. Bul. 161. 1913.

Effect of Rapid Cooling on the Bacterial Content of Milk

Trial Number	Pasteurization Exposure	Bacteria per CC.		
		In Raw Milk	In Uncooled Milk	In Cooled Milk
1	62.8° C. (145° F.)—30 min....	186,000	8,600	8,500
2	62.8° C.—30 min.	233,000	1,470	2,160
3	71.1° C. (160° F.)—30 min...	400,000	1,880	1,950
4	71.1° C.—30 min............	1,350,000	1,750	1,700

From these results, it is evident that the rapid cooling, following pasteurization at either of the temperatures used, did not cause a decrease in the number of bacteria present.

Effect of increased holding at pasteurization temperatures. Retaining milk for unusually long periods at the temperatures used with the holding method of pasteurization does not ordinarily result in a bacterial count significantly lower than that secured with normal holding. It appears that, if organisms can resist the usual holding temperature and time, they can also survive this temperature for a considerably longer period.

Increase in the use of pasteurization. When pasteurization was introduced into the milk industry the main object was to improve the keeping quality of the product and, accordingly, the process was sometimes carried out surreptitiously. However, a clear understanding of what pasteurization accomplishes soon established confidence in it. As a result of the continued tracing of epidemics to dairy products and the finding of pathogens, especially *M. tuberculosis*, in these materials, certain of the agencies supervising milk supplies began to require the pasteurization of all milk, except that produced under the most careful conditions. There has also been a great increase in the use of the process in cities where it is not compulsory, due to an understanding of its advantages by the milk dealers. Pasteurized milk is now available in many of the smaller towns and sometimes makes up a considerable percentage of the total supply. When a milk-borne outbreak of some disease occurs there is often an immediate demand for pasteurization in the locality involved. Many persons who are interested in the safety of the food materials they consume insist on pasteurized milk and cream, and this demand is being stimulated, in certain instances, by well planned advertising which points out the value of pasteurization. The public generally is beginning to appreciate that, with milk which is shipped for long distances and which comes in contact with many persons, any one of whom may contaminate it with pathogenic organisms, the situation is very different than when family and neighborhood cows were common and the consumer could easily learn how the milk was produced and handled.

In a number of instances an attempt has been made to satisfy a demand for pasteurization by advocating the tuberculin testing of the cows producing the milk. It should be recognized that, while tuberculous cows are highly objectionable, they constitute only one of the dangers from milk and that tuberculin testing gives no protection as far as milk-borne diseases other than tuberculosis are concerned. Proper pasteurization frees milk from all the pathogens that are to be expected in it and is, accordingly, more inclusive than any system of cattle testing. Regulations which require either pasteurization or tuberculin testing do not take into account the accomplishments of the two procedures. Tuberculin testing is not a substitute for pasteurization and while it is highly desirable for all milk, whether it is to be consumed raw or heated, it accomplishes much less, in the way of protection, than pasteurization.

Inefficient pasteurization. In the past there has been a great deal of inefficient pasteurization, due either to the selection of unsatisfactory exposures or to a failure to secure the exposures desired. Very often such pasteurization gives milk a satisfactory keeping quality so that its inefficiency is not readily recognized, except by bacterial counts or by a consideration of both the exposures used and those necessary for the destruction of pathogens. Pasteurization that is inadequate is thoroughly misleading since the informed consumer expects a protection that is not given.

In the epidemics due to pasteurized dairy products, the difficulty has sometimes occurred through the use of exposures that are too low, while in the other instances infection has followed the pasteurization. The inadequate exposures have been much more frequent with flash pasteurization than with the holding process because of the difficulty of providing a reasonable margin of safety without seriously interfering with the flavor and creaming ability of milk. In the past the regularly used exposures have sometimes been so low that, had pathogens been present, their destruction would not have been accomplished.

The bacteria which survive pasteurization. During the period when pasteurization was being rapidly extended in the field of dairying, the prevailing idea was that the organisms which survived the process were largely of the spore forming type. This view came, in part, from the studies carried out on the organisms present in milk heated to temperatures much higher than those employed in pasteurization. Investigations have shown, however, that a variety of non-spore forming bacteria are capable of resisting the exposures accepted as satisfactory and that these constitute a much larger percentage of the flora of pasteurized milk than do the spore formers. Ayers and Johnson [63] found that, of 225 unselected cultures which survived pasteurization at 62.8° C. (145° F.) for 30 minutes, only 3 or 1.35 per cent formed spores. The non-spore forming bacteria, accordingly, play an important role in the bacterial changes which go on in pasteurized milk and cream.

Organisms producing acid in milk, either with or without coagulation, are especially prominent among those surviving pasteurization. Ayers and Johnson [64] noted that the percentage of the total flora made up of these two groups was greater in milk pasteurized at 62.8° C. (145° F.), 71.1° C. (160° F.) or 76.7° C. (170° F.) for 30 minutes than in raw milk; at still higher exposures the acid coagulating group disappeared and the acid non-coagulating group was greatly reduced in importance. Acid producing organisms can ordinarily be isolated from pasteurized milk by allowing it to stand, preferably at from 30° to 37° C. (86° to 98.6° F.), until considerable growth has occurred and then plating and picking colonies; unless precautions against contamination after pasteurization are taken, the organisms secured may be types that gained entrance subsequent to the heating.

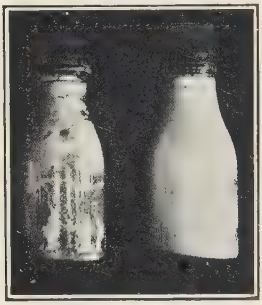


Fig. 23.—Types of curd, highly heated milk (left) and raw milk (right) after two days at room temperature.

Some of the lactic acid bacteria are capable of resisting comparatively high exposures to heat. Ayers and Johnson [65] isolated a lactic acid organism from commercially pasteurized milk which, when heated in Sternberg bulbs for 30 minutes, required 74.4° C (166° F.) in broth and 75.6° C. (168° F.) in milk for its destruction. When heated for 10 minutes in milk the temperature required was 77.8° C.

---

[63] See ref. 62.

[64] See ref. 62.

[65] U. S. D. A. B. A. I. Bul. 126. 1910.

(172° F.). Later, these investigators[66] found a culture which survived 30 minutes at 79.4° C. (175° F.) when a broth culture was heated in a Sternberg bulb; from one sample of milk a number of cultures were secured which survived 87.8° C. (190° F.) but not 93.3° C. (200° F.) for 30 minutes when a small amount of culture was transferred to a tube of milk and the milk heated in a water bath.

One of the lactic acid organisms which is commonly present in pasteurized milk is *S. thermophilus.* This species is closely related to *S. lactis* and differs from it primarily in the temperature relationships. *S. thermophilus* is rarely encountered in the isolation of lactic acid organisms from raw milk, either sweet or sour, due probably to its slow growth in competition with *S. lactis* at the common holding temperatures. In the work of Evans, Hastings and Hart[67] on the bacteria concerned in the flavor production in cheddar cheese, *L. casei* was regularly present in cheese made from pasteurized milk and this suggests that the organism resists pasteurization exposures; however, it usually developed more slowly in pasteurized milk cheese than in raw milk cheese so that the heating may have killed some of the cells. Hammer and Baker[68] found that, with a heating period of 30 minutes, a considerable number of the *S. paracitrovorous* cultures studied survived 60° C. (140° F.), while a somewhat smaller number survived 65° C. (149° F.); one culture survived 75° C. (167° F.).

Although various studies have indicated the Escherichia-Aerobacter organisms are not very resistant to heat this type sometimes survives pasteurization. Ayers and Johnson[69] studied the heat resistance of 174 cultures isolated from cow feces, human feces, flies, milk, cream and cheese, using a heating period of 30 minutes, and found that 95 or 54.5 per cent survived 60° C. (140° F.), while 12 or 6.89 per cent survived 62.8° C. (145° F.). These results show that gas forming lactic acid organisms may be present in properly pasteurized milk and that the finding of such organisms can not be taken as an indication of unsatisfactory heating. Swenarton[70] noted a correlation between the Escherichia-Aerobacter content of pasteurized milk and the pasteurization procedure, as indicated by the control charts; the charts from plants whose milk was high in these organisms showed improper heating or irregularity of procedure.

[66] See ref. 62.

[67] Jr. Agr. Res. 2. P. 167. 1914.

[68] Ia. Agr. Expt. Sta. Res. Bul. 81. 1923.

[69] Jr. Agr. Res. 3. P. 401. 1915.

[70] Jr. Bact. 13. P. 419. 1927.

The cocci are an important morphologic type among the non-spore formers surviving the usual pasteurization exposures and the heat resistance of these organisms is evident from various investigations. Ayers and Johnson[71] studied 139 cultures of streptococci isolated from cow feces, the udder and the mouth of the cow, and from milk and cream and found that, with 30 minutes' exposure in milk, 89 or 64.03 per cent survived 60° C. (140° F.), 46 or 33.07 per cent survived 62.8° C. (145° F.), while 3 or 2.56 per cent survived 71.1° C. 160° F.); all were destroyed at 73.9° C. (165° F.). The 22 cultures which formed long chains were much less resistant to heat than the 117 cultures which did not. Hammer and Trout[72] noted that cocci forming yellow colonies were often present on plates poured with dairy products, especially those that had been subjected to heat, and that these organisms ordinarily resisted the usual pasteurization exposures for market milk; they were resistant in skim milk, whole milk, cream, bouillon and ice cream mix, but the resistance in milk was decreased in the presence of sufficient acid to cause coagulation. The yellow cocci studied belonged to several different types. Brannon and Prucha[73] investigated a number of bacterial species for their resistance to pasteurization at 62.5° C. (144.5° F.) for 30 minutes and found that, among the non-spore formers, a sarcina was the only one surviving.

At exposures considerably above those usually employed for the pasteurization of milk, peptonizing bacteria make up a large percentage of the total organisms. This is presumably due to the almost complete destruction of the non-spore forming types, so that the spore formers, many of which are peptonizers, are the principal organisms remaining.

The resistance of an organism to heat is not a sharply defined character that can be exactly determined. The individual organisms in a pure culture may vary a great deal in their resistance and, ordinarily, some few cells that retain their vitality at exposures much higher than those required for the destruction of the others enable the culture to survive rather severe conditions. Considerable variation is also encountered in the resistance of different cultures belonging to the same species and this accounts for a species sometimes surviving pasteurization and sometimes not.

Pin-point colonies from pasteurized milk. The agar plates poured

[71] Jr. Agr. Res. 2. P. 321. 1914.

[72] Jr. Dairy Sc. 11. P. 18. 1928.

[73] Jr. Dairy Sc. 10. P. 263. 1927.

with pasteurized milk and other dairy products sometimes show very small colonies that are often referred to as pin-point colonies. These may be large enough to be readily recognized with the unaided eye but, in some instances, observations with a hand lens are necessary before they can be definitely established as colonies. The presence of these small colonies is not limited to plates poured with pasteurized products, but they have been most often noted in the examination of pasteurized milk and, occasionally, have been responsible for excessive counts on this product.

There are various causes for the small size of colonies on agar plates. If the dilution is not high enough the crowding results in a poor development. A medium that is unsatisfactory, either because of the general composition or the reaction, may be responsible for small colonies. Fay [74] noted that the sucrose present in the plates poured with ice cream made it possible for organisms to develop that could not grow without the sucrose and, later, this same investigator [75] found that the small amount of lactose carried over in a 1/100 cc. dilution of milk was a factor in enabling certain organisms to grow on the plates. An observation reported by Cooledge [76] illustrates the influence of the reaction of the medium on colony development. The average bacterial count of four samples of milk plated with a medium having a reaction of pH 6.5 to 6.6 was 15,400 per cc., while with agar from the same lot adjusted to pH 7.3 the average count was 317,000. Cooledge also noted an instance in which the reaction of standard agar plates (pH 6.6) containing over 20,000 organisms of an alkali type was so changed by the organisms that they developed pin-point colonies while, in the next dilution, the smaller number of organisms was unable to overcome the reaction of the medium and there was no visible growth.

An important cause of pin-point colonies is undoubtedly the presence of organisms which develop poorly on agar even under the most favorable conditions. Ayers and Johnson [77] isolated an organism, which they designated *Lactobacillus thermophilus,* that was responsible for pin-point colonies on plates poured with milk from a certain dairy plant. Thirty-seven of 39 cultures isolated were of this type, which shows that the pin-point colonies were largely due to one species. It was also secured from pasteurized milk from another

[74] Jr. Bact. 11. P. 96. 1926.

[75] Jr. Bact. 13. P. 347. 1927.

[76] Absts. Bact. 8. P. 20. 1924.

[77] Jr. Bact. 9. P. 285. 1924.

dairy. In the investigation reported by Fay,[78] three main morphologic types of organisms were responsible for pin-point colonies; all of them were gram positive, non-spore forming, short rods or cocci. The pin-point colonies noted on routine plates poured with pasteurized milk are commonly streptococci that grow and produce acid in milk only slowly. Their slow development in this medium may be typical of their rate of growth under all conditions and the small colonies result in this way.

Pin-point colonies have been of interest chiefly because of their occurrence in plates showing a high count on pasteurized milk. Jacobsen[79] reported that two milk plants, both efficiently equipped and operated and receiving milk of the same general quality, obtained dissimilar results from the pasteurization, one having an average bacterial count on the heated milk of 32,000 per cc., while the other had a count of 176,000. The examination of the milk from individual shippers supplying the second plant showed that milk from one farm contained large numbers of a heat resistant organism. A sample of this milk had a count of 197,500 per cc. before pasteurization, while after heating in a bottle at 62.2° C. (144° F.) for 40 minutes, the count was 246,000. Yates[80] noted that unusually high counts secured on Kansas City milk, mainly with pasteurized lots, were uniformly due to the presence of pin-point colonies. Results showing a correlation between excessive counts on pasteurized milk and pin-point colonies on the plates have been reported by various investigators. Fay[81] observed pin-point colonies in the low dilution plates made in the bacteriological analysis of ice cream.

The data available indicate very definitely that organisms may develop in milk at pasteurizing temperatures and certain investigations, such as those of Taylor[82] and Hungerford and Harding,[83] indicate that such growth may occur in milk while it is in pasteurizing equipment. Some of the organisms giving pin-point colonies are evidently incapable of growth at pasteurizing temperatures, although they may tolerate such temperatures for considerable periods. Fay[84] studied cultures giving small colonies which repeatedly failed to grow at 63° C. (145.4° F.); usually, however, they survived an exposure of one or two hours at this temperature.

Organisms that will grow at pasteurizing temperatures have been found in raw milk by various investigators. Adams and Harding[85] reported them in 28.2 per cent of the samples of common milk examined, in 43.7 per cent of the class A samples and in 40.4 per cent of the certified samples. It should be noted that some of the organisms capable of growth in milk at pasteurizing temperatures do not form pin-point colonies on plates so that there is no complete correlation between the two characters.

---

[78] See ref. 75.

[79] Absts. Bact. 2. P. 215. 1918.

[80] Absts. Bact. 7. P. 24. 1923.

[81] See ref. 74.

[82] Absts. Bact. 8. P. 17. 1924.

[83] Absts. Bact. 8. P. 17. 1924.

[84] See ref. 75.

Bacterial changes in pasteurized milk. In milk efficiently pasteurized and properly cared for subsequent to the heating, bacterial changes occur much more slowly than in raw milk from the same lot. The increase in the time that the pasteurized milk will remain unaltered can not be predicted, because of the various influencing factors, but it is sufficient so that, with reasonable holding conditions, the milk will be used before changes occur in it. If the pasteurized milk is seriously contaminated following the heating, the keeping quality may be very poor, often not much better than that of raw milk.

The type of fermentation that takes place in pasteurized milk as a result of bacterial action shows certain variations, just as does the type of fermentation in raw milk. It is influenced by various factors, such as the contamination subsequent to the heating, the pasteurization exposure used, the holding temperature and the original flora. In general, the holding of pasteurized milk under the usual conditions results in either the development of acid or sweet curdling. Acid formation is not noted as regularly as with raw milk and, when it occurs, microscopic examination or plate cultures often show a variety of organisms, instead of the predominance of streptococci that is so common when raw milk develops acid. Although large numbers of acid forming organisms may be present in pasteurized milk their action is slower than the action of certain of the acid formers in raw milk and a rapid increase in acidity is not noted, unless contamination subsequent to the heating has occurred.

ADVANTAGES OF PASTEURIZATION

The advantages of proper pasteurization are (1) reduction of the spread of pathogens through milk, (2) reduction in infant mortality and (3) improvement in the keeping quality of milk.

Reduction of the spread of pathogens through milk. The exposures used in pasteurization have been selected so that pathogens present in milk or cream will be destroyed. The process is no guarantee against future infection, however, and a number of epidemics have been due to the contamination of milk after heating. It should be noted that, in milk-borne epidemics, infection has most frequently occurred on the farms; Godfrey[86] concluded from a study of data dealing with a large number of epidemics that 85 to 90 per cent were due to farm infection. Pasteurization, when properly carried out, establishes the safety of milk or cream up to the time of heating and infection subsequent to this must be guarded against by proper equipment and satisfactory methods of handling.

---

[85] Absts. Bact. 8. P. 18. 1924.

The actual decrease in milk-borne diseases as a result of pasteurization is extremely difficult to determine because other factors have been influencing the same diseases; for example, the decrease in typhoid fever during the last two decades has been pronounced and the pasteurization of milk has played a part in this, but such factors as improvements in water supplies, better sewage disposal, immunization, etc., have also been operative and it is impossible to ascertain the influence of each of these separately. The value of pasteurization is directly shown by the instances in which raw milk from a certain source has caused an epidemic, while pasteurized milk from the same source has proved safe. It is a common practice to begin pasteurization as soon as a raw milk supply is under suspicion and many epidemics have been stopped by this procedure. Evidence of the value of pasteurization is also provided by the definite decrease in milk-borne diseases that has occurred in certain cities. It should be recognized, however, that more careful methods of producing and handling milk have been a factor in decreasing the spread of diseases through it and that pasteurization can not be given the entire credit.

The great majority of the milk-borne epidemics have involved raw milk supplies and, while the exact significance of this is difficult to determine because data on the comparative consumption of raw and pasteurized milk have not been available, it is very suggestive. Milk-borne outbreaks have been especially numerous in countries, such as the United States, where the consumption of milk in a raw state has been common.

Reduction in infant mortality. In the feeding of infants, milk that has been pasteurized, either in plants or in homes, has been extensively used. While the effect of the destruction of large numbers of organisms, other than the specific pathogens, on the infant mortality can not be definitely determined, the evidence indicates that it has been a factor in decreasing deaths due to intestinal disturbances of various types. The production methods followed with much of the milk used in cities and the time involved in getting it to the consumers suggest that large numbers of organisms would be present if pasteurization were not practiced and it seems possible that, in some instances, these would be harmful to infants ingesting them.

---

[86] Nation's Health. 5. P. 1. 1923.

Improvement in the keeping quality of milk. The necessity of delivering milk with a reasonably good keeping quality is appreciated by all experienced milk dealers. A product that readily sours or undergoes other undesirable changes is certain to result in dissatisfaction and complaint among the consumers and is very likely to cause the dealer a loss of patronage. Milk should have a keeping quality that will enable it to remain sweet for at least 36 hours under the usual holding conditions and, while this period may vary somewhat with the season, it should always be long enough so that the milk will be consumed before it undergoes changes.

Pasteurization is extremely valuable in giving milk a satisfactory keeping quality. In many of our cities the consumers are so far from the producing farms that the milk is from 24 to 48 hours old when it is delivered and, if it is to keep in a good condition 36 hours longer at the usual holding temperatures, some such treatment as pasteurization is necessary. Even in smaller towns, where the milk is delivered at an age not exceeding 12 to 18 hours, the keeping quality may cause a great deal of annoyance during the warm months unless pasteurization is employed. The difficulty along this line is increased when the dealer has little if any control over the methods used in producing the milk and so can not insist on changes that will keep down the number of bacteria.

During the summer season, when most of the trouble with keeping quality is experienced, there is often a decreased milk flow so that the dealer may not have available a supply which permits the refusal of the milk of the more careless producers. The danger of some unusual contamination suddenly giving the milk a poor keeping quality and thus causing complaints, has forced many dealers to employ pasteurization for their own protection. With the success of a business depending on the satisfaction given by the product supplied, some safeguard is advisable and proper pasteurization provides a valuable form of insurance.

In the past, milk inspectors have sometimes maintained that the employment of pasteurization to improve the keeping quality of milk is an unfair practice and that the only legitimate use is to destroy disease producing organisms. The primary object of pasteurization should be to provide safe milk but the other advantage of the process need not be ignored. With customers justifiably demanding milk that keeps well and with a complex milk distributing system over which the dealer does not always have complete control, the use of pasteurization as a help in the solution of the keeping quality problem is very logical.

OBJECTIONS THAT HAVE BEEN RAISED TO PASTEURIZATION

Since the introduction of pasteurization various objections to the process have been raised from time to time. These have often come from persons who prefer to sell milk in a raw condition, because of the expense of installing pasteurizing equipment, and attempts to establish regulations requiring pasteurization commonly result in protests, especially from distributors whose supplies are so small that the expenditures for equipment must be kept very low. Occasionally objections to pasteurization have come from physicians and others who prescribe the type of milk to be used by infants and invalids.

At one time there may have been some foundation for certain of the objections that have been raised to pasteurization but improvements in the methods, in the quality of the raw milk used and in the general understanding of the process have removed any basis for most of them and, at present, the objections come largely from persons who do not have a clear understanding of what the process accomplishes.

Some of the more frequently mentioned objections to pasteurization follow:

1. The organisms developing in pasteurized milk form harmful products. This idea appears to have been based on the work of Flügge,[87] who produced illness in puppies by feeding them milk cultures of organisms secured from boiled milk. The results obtained are not directly applicable to pasteurized milk, since the organisms were isolated from milk heated to much higher temperatures than those used in pasteurization, but the types which survive boiling would also be expected in pasteurized milk. The development of toxic products in milk held for extended periods, with either pure or mixed cultures, is quite

---

[87] Ztschr. f. Hyg. 17. P. 272. 1894.

different than such a development in milk held under the usual conditions. With long holding at temperatures favorable for bacterial growth either raw or pasteurized milk may form products that are toxic for experimental animals, but the milk ordinarily has undergone conspicuous changes and there is little likelihood that it would be used for food.

2. Infants do not develop as well on pasteurized milk as on raw milk. The claim is occasionally made that pasteurized milk is not satisfactory for infants. The best argument against this is the large number of well developed children that have been fed this product. Careful observations in various cities have shown that many infants make entirely normal gains on pasteurized milk. Because of differences in infants, sweeping conclusions should not be drawn and it may be that an occasional infant assimilates raw milk better than pasteurized, just as some infants use sour milk more satisfactorily than sweet.
3. Products of bacterial growth are not destroyed. It is probably true that certain toxic products formed by the growth of bacteria in milk resist pasteurization exposures but, if the same milk were consumed in a raw state, the toxic products would be equally or even more active. Since the products referred to are not the result of pasteurization but develop before it is used, the process can not be held accountable for them. Milk in which toxic products are present as the result of bacterial action must contain large numbers of organisms and these indicate unsatisfactory methods of production or handling; such milk is not satisfactory for consumption in either a raw or pasteurized condition.
4. Pasteurization may be used to cover up dirty milk. In some instances pasteurization has undoubtedly been employed to give milk, which has undergone extensive bacterial growth, a keeping quality that makes it marketable. However, milk that is to be pasteurized requires the same supervision as milk that is to be sold raw and pasteurization can not replace unsatisfactory methods of production and holding. With suitable standards for the milk coming to pasteurizing plants the producers soon appreciate that careful methods are necessary. The improper use of pasteurization can not be blamed on the process and should be stopped through supervision of the supplies intended for heating.
5. Milk enzymes are destroyed. Some of the normal milk enzymes

are partially or completely destroyed by the usual pasteurization exposures. There is no evidence, however, that these enzymes play a part in the digestion process and any influence that pasteurization has on them is likely to be unimportant from the standpoint of the person consuming the milk.

6. Influences the composition of milk. The investigations that have been carried out indicate that pasteurization at the usual exposures has little effect on the composition of milk. Rupp[88] made a series of analyses on milk samples pasteurized at various temperatures for 30 minute periods. Milk heated to 62.8° C. (145° F.) did not undergo an appreciable change. The soluble phosphates of lime and magnesia did not become insoluble. At 68.3° C. (155° F.) the quantities of phosphoric acid, lime and magnesia in the serum of raw and of pasteurized milk were practically the same. The albumin did not coagulate at 62.8° C. but, at 65.6° C. (150° F.), 5.75 per cent was rendered insoluble and, as the temperature was increased, the amount of coagulated albumin increased. The time required for coagulation by rennin was slightly less in milk pasteurized at temperatures up to 65° C. (149° F.) than in raw milk, but at 70° C. (158° F.), it was slightly increased and at 75° C. (167° F.) it was almost doubled.
7. Influence on vitamines. With the rather recent appreciation of the value of vitamines in the diet the question of the influence of pasteurization on these has become of importance. Various investigations[89] have shown that pasteurization has no detectable influence on vitamines A and B while C is probably influenced to a certain extent, either through the action of the heat or through the increased opportunity for oxidation during pasteurization. Vitamin C is not abundant in milk and is easily supplied in such materials as orange juice or the juice of canned tomatoes so that the effect of pasteurization on it is not serious.

At the present time there are no justifiable objections to pasteurization, when the process is properly carried out on milk that is of a satisfactory quality before heating, and the process is widely recognized as a very valuable aid in the delivery of a safe milk. When an attempt is made to prevent the contamination of milk from all of the sources that may yield pathogenic organisms, the expense of

[88] U. S. D. A. B. A. I. Bul. 166. 1913.

[89] Am. Jr. Pub Health. 12. P. 908. 1922.

production is greatly increased and the cost limits the use of the product. Milk of this quality is, for the most part, available only in the larger cities and even there rarely constitutes more than 1 or 2 per cent of the total milk supply. The general acceptance of pasteurization by city health departments affords ample proof of the value of the process, as judged by those who are in the best position to appreciate what it accomplishes. The rapid increase, during recent years, in the amount of milk pasteurized, even in cities where pasteurization is not compulsory, is another convincing argument.

The importance of properly carrying out the pasteurization must be definitely recognized. In the past exposures that are not at all satisfactory have been used, especially with the flash method, and often serious contamination from coolers, bottle fillers, etc., followed the heating. Insufficient cooling after pasteurization is another common defect in the handling of milk, particularly in the smaller plants. Because of the unsatisfactory methods used the milk from some plants has shown a higher count after pasteurization than before and, under such conditions, the process is of little value. A better understanding of the pasteurization process by milk plant operators, the invention of more efficient pasteurizing equipment and the educational control work carried out by health departments are undoubtedly tending to rapidly do away with unsatisfactory conditions but the necessity for care and accuracy must be appreciated.

THE ELECTRICAL TREATMENT OF MILK

The studies that have been carried out on the effect of an electric current on bacteria have included attempts to largely destroy the organisms in milk by the passage of a current through it. An important series of investigations along this line was begun in Liverpool, in 1911, and has involved various methods of treating the milk. The use of a rapidly alternating current was found the most successful and formed the basis of the method eventually selected. The milk was passed through a long tube in which three electrodes were placed, the central electrode being connected with one pole and the two outer electrodes in parallel with the other pole. In 1914, Beattie [90] reported data obtained on milk treated in a plant handling from 100 to 125 gallons of milk daily. The method destroyed *M. tuberculosis* in the milk and, in a series of 15 daily examinations, 99.93 per cent of the total bacteria were killed. The treated milk kept sweet for at least three or four days and the taste was unaltered. In one trial for which data were given, the voltage varied from 3,650 to 4,200, the amperage from 2.02 to 3.25 and the developed temperature from 60° to 64° C. (140° to 147.2° F.). Since the exposure to heat was for only a few seconds, the temperature influence was considered negligible. Certain investigators have, however, considered the temperature increase the important thing in the destruction of organisms. Beattie and Lewis [91] carried out tests intended to decide this point and concluded that the heat is not the main factor in the destruction, but that the electric current is an important agent. When subjected to the method of treatment used, bacteria were destroyed at a temperature much below their thermal death point.

---

[90] Rpt. City Bacteriologist, City of Liverpool, on Electrical Treatment of Milk. 1914.

Anderson and Finkelstein [92] studied a process for the electrical treatment of milk in which the milk was heated with steam to 40° C. (104° F.), delivered to a supply tank and then run to the machine itself, which was made up of units each consisting of five porcelain cups so arranged that the milk flowed from one to the other. The first cup had about twice the capacity of the others. In each of the four small cups there was an electrode, which was an insulated rod terminating in a copper disc. The current was completed by the stream of milk. The voltage was 2,300, the amperage 14 and the frequency 25. The milk was raised to a temperature of about 70° C. (158° F.) in the machine and then went to a cooler. The method gave a very satisfactory reduction of the total count in good quality milk and a satisfactory reduction in poor quality milk; the treated milk kept well. The precipitation of albumin was slight, if it occurred at all, and the cream line was not interfered with. The destruction of bacteria appeared to be due to the heat produced.

Prescott [93] investigated an installation for treating milk in which the milk was heated to about 47.8° C. (118° F.) in a regenerative cooler and then subjected to an alternating current of 220 volts, 60 cycles. In the treating chamber the milk was raised to from 70° to 71.1° C. (158° to 160° F.) and was then cooled to a low temperature. The milk did not have a cooked taste, the cream line was well marked and the keeping quality was excellent. During the winter, when the raw milk gave a count of less than 200,000 bacteria per cc., the percentage reduction in the organisms approximated 90 per cent while in the warmer months, with the raw milk running from 500,000

[91] Jr. Hyg. 24. P. 123. 1925.

[92] Jr. Dairy Sc. 2. P. 374. 1919.

[93] Am. Jr. Pub. Health. 17. P. 221. 1927.

to 600,000 per cc., the reduction varied from 97 to 98.5 per cent. No Escherichia-Aerobacter organisms were found in the treated milk. Under the recommended commercial conditions of 71.1° C., 220 volts, 60 cycles, *M. tuberculosis* was destroyed.

EFFECT OF ULTRA-VIOLET RAYS ON THE BACTERIA IN MILK

The attempts that have been made to destroy the organisms in milk have included trials with ultra-violet rays.

Houghton and Davis[94] investigated the action of ultra-violet rays on the bacteria in various materials, when these flowed through a trough exposed to a lamp. A comparatively low reduction in the organisms in milk was secured. It was noted that the ultra-violet rays had a tendency to kill the undesirable organisms present, leaving *S. lactis* in the majority. There was no perceptible change in the taste of the milk as a result of the exposure.

Ayers and Johnson[95] carried out a series of tests in which milk was exposed in various ways to ultra-violet rays generated by a quartz mercury vapor lamp operating on a 220 volt circuit and taking 3.5 amperes. The most satisfactory method of exposure found was over two revolving drums, the tops of which were four inches below the light tube. The milk picked up by one drum was exposed, then collected by a scraper, after which it was picked up by the second drum and again exposed; finally it was collected by a second scraper. When the milk was exposed in thin layers there was a marked reduction in bacteria and this was independent of the action of the heat, since the temperature of the exposed milk was never above 30° C. (86° F.). The two most important factors in the success of the method were the thickness of the layer of milk and the length of the exposure.

A study of two samples of exposed milk indicated that the rays did not have a selective action in the destruction of organisms, although there was a greater effect on vegetative cells than on spores. When milk was exposed under conditions suitable for a satisfactory reduction of bacteria by the rays, an abnormal, disagreeable flavor was produced. Under similar conditions of exposure there seemed to be less reduction of organisms in 15 per cent cream than in milk and this was considered to be due to the thicker layer in which the cream was carried up by the drums.

[94] Am. Jr. Pub. Health. 4. P. 224. 1914.

[95] Centbl. f. Bakt. 2 Abt. 40. P. 109. 1914.

CHAPTER IX

MILK ENZYMES

At the time milk leaves the udder it normally contains certain enzymes. None of these bring about rapid changes in milk and so can not be considered important from the standpoint of the keeping quality of the product. Some of the enzymes, however, are of significance through the part they play in the changes, either desirable or undesirable, that occur in milk derivatives held for rather long periods, while others are of importance because their action is involved in certain tests that are applied to milk or the products made from it. The milk enzymes which appear at present to be of the greatest importance are galactase, lipase, catalase and peroxidase. Some of the others that have been reported are reductase, lactase, diastase, and salolase, although the evidence with reference to the presence of certain of these is by no means conclusive.

The enzymes of milk require consideration along with the bacteria in dairy products because of the similarity and relationship of enzymatic and bacterial action. Both may bring about the same general types of changes in certain of the constituents of milk and both are greatly influenced by the temperature; this relationship is what would be expected when it is appreciated that the action of micro-organisms is brought about through the enzymes elaborated.

Differentiation of changes due to enzymes and to micro-organisms. In the study of the changes due to enzymes the action of micro-organisms is commonly prevented by some such reagent as chloroform, ether or formaldehyde. It is advisable to examine, both culturally and microscopically, the material being investigated for changes in order to be certain that micro-organisms are not developing. The importance of this was clearly shown through the isolation by Babcock and Russell[1] of an organism that possessed a definite ether tolerance; the character was, however, lost during a series of transfers on agar.

---

[1] Wis. Agr. Expt. Sta. 14th An. Rpt. P. 211. 1897.

Rahn [2] has pointed out that the changes produced by enzymes and by bacteria differ in rate. With enzymes the active agent does not increase and, accordingly, the rapidity of the process does not, but tends to fall off with time while, with bacteria, the active mass increases and, as a result, the rate of the process does also until conditions become unfavorable for the organisms. In impure cultures the influence of one organism on another may greatly modify the changes occurring.

Bacteria sometimes influence the action of enzymes through their effect on the reaction. This is illustrated by the slower action of galactase in an acid than in a neutral or alkaline medium.

Galactase. In 1897, Babcock and Russell [3] reported that in milk to which chloroform or ether had been added coagulation and an increase in the amount of soluble nitrogen occurred. Their experiments indicated that the precipitation of the casein was influenced by the temperature of holding more than anything else. It was found possible to make enzyme preparations that had a curdling as well as a digesting action on milk. Babcock, Russell and Vivian [4] proposed the name galactase for the coagulating and digesting enzyme and presented data suggesting that the curdling action is independent of the peptonizing action. Their results further show that the activity of the enzyme is materially retarded in the presence of acid and this was interpreted as indicating a relationship to the tryptic rather than the peptic type of enzyme. Babcock, Russell, Vivian and Hastings [5] studied the action of galactase in comparison with various enzymes and cultures of different types of bacteria from the standpoint of the decomposition products in milk. They concluded that the products formed by galactase approach more nearly those produced by the peptonizing bacteria than by any of the ferments of animal origin.

Galactase can be demonstrated in milk by treating a sample with chloroform or ether, stoppering tightly to prevent loss of the reagent, and then holding at a favorable temperature, such as 37° C. (98.6° F.). The milk usually forms a soft curd in from one to three weeks; the curd gradually acquires a somewhat transparent appearance, due presumably to the proteolysis taking place. Soluble nitrogen determinations on the fresh and curdled milk show the extent of the nitrogenous decomposition. Galactase can also be demonstrated at room temperature but it is less active there than at 37° C.

---

[2] Mich. Agr. Expt. Sta. Tech. Bul. 5. 1911.

[3] Wis. Agr. Expt. Sta. 14th An. Rpt. P. 161. 1897.

[4] Wis. Agr. Expt. Sta. 15th An. Rpt. P. 77. 1898.

[5] Wis. Agr. Expt. Sta. 16th An. Rpt. P. 157. 1899.

The work of Babcock, Russell and Vivian[6] has shown the presence of galactase in the milk of various species of animals, including man, sheep, goat, pig, horse, burro and a half-breed buffalo. Thatcher and Dahlberg[7] found that during the separation of cows' milk galactase was highly concentrated in the slime and slightly concentrated in the cream.

Galactase[8] is active in the presence of chloroform, ether, benzol and toluol but strong disinfectants like corrosive sublimate, formaldehyde, phenol and carbon bisulfide destroy it. According to Thatcher and Dahlberg,[9] however, 1 per cent chloroform in skim milk or buttermilk entirely inhibited proteolysis while smaller amounts permitted it. Proteolysis is more active in a slightly alkaline or neutral reaction but also occurs when some acid is present. The protein decomposition brought about by galactase goes to the stage of amino acids. Some of the earlier work suggested that ammonia was formed but this has not been confirmed by the later studies.

Babcock, Russell and Vivian[10] found that heating enzyme solutions for 10 minutes at 76° C. (168.8° F.) sufficed to destroy galactase and that 10 minutes at 71° C. (159.8° F.) materially reduced its activity. Rogers, Berg and Davis[11] reported that with the continuous method of pasteurization galactase was much weakened by temperatures between 71° C. and 77° C. (170.6° F.) but was not destroyed at 93° C. (199.4° F.), the highest temperature employed. Galactase appears to be the most resistant to heat of the enzymes normally present in milk.

Galactase is believed to be an important factor in the ripening of various hard cheeses, its role being to aid in the breaking down of the complex nitrogenous constituents. This decomposition is necessary for the proper development of texture and flavor, and chemical examinations show that it involves a considerable portion of the protein originally present. Because of the presence of curd in butter, the action of galactase must also be considered as a possible factor in the deterioration of this product. Thatcher and Dahlberg[12] secured a uniform and measurable proteolytic action from two samples of

[6] Wis. Agr. Expt. Sta. 15th An. Rpt. P. 93. 1898.

[7] Jr. Agr. Res. 11. P. 437. 1917.

[8] See ref. 4.

[9] See ref. 7.

[10] See ref. 4.

[11] U. S. D. A. B. A. I. Cir. 189. 1912.

[12] See ref. 7.

fresh dairy butter but none from a sample of fresh creamery butter. A sample of stored dairy butter and another of stored centralizer butter gave a greater increase in the soluble nitrogen than the fresh butter. These investigators noted that sodium chloride greatly influenced the activity of galactase, 15 per cent in skim milk entirely preventing proteolysis, which led them to conclude that galactase can not act in normal salted butter because of the salt content.

The name galactase for the curdling and proteolyzing enzyme of milk has resulted in a great deal of confusion since, under the present system of nomenclature, it suggests an action of a very different type.

Lipase. The presence in milk of an enzyme which splits fat into fatty acids and glycerol has been investigated rather extensively. The usual method of study has been to employ either a simple ester or a heated fat, allow the material suspected of containing the enzyme to act on this in the presence of water and a reagent which restrains bacterial growth and then titrate for an increase in acidity indicative of hydrolysis. Various restraining reagents have been used, especially chloroform and ether; Palmer[13] employed formaldehyde in the proportion of 1 to 1,500 or 2,000 because it has no detrimental effect on the activity of animal lipase while chloroform retards this action. It should be noted that the hydrolysis of a simple ester by an enzyme does not imply that a fat will be hydrolyzed.

The evidence with reference to a lipase in milk is conflicting. Some of the early investigators reported the presence of this enzyme but the recent studies of Palmer,[14] which were planned to overcome some of the objections to the more usual methods, indicate that cows' milk does not normally contain an active lipase. Rice and Markley,[15] however, recently found a lipase in milk. They used a high fat content cream as a substrate and added sufficient sucrose to saturate it and any water added later; two parts of sucrose to one part of water were used and the mixture of cream and sugar boiled to facilitate solution and to destroy the enzymes in the cream. The material suspected of containing lipase was then added and titrations made at once and again after holding.

Rogers[16] has reported data indicating the activity of lipase in butter. He compared the increase in acidity in unheated and heated butter, and found that in the unheated material there was considerable increase in acid while in the heated material, in which the enzymes had presumably been destroyed, the change in acidity was negligible. Thatcher and Dahlberg[17] studied six samples of butter and found the lipase activity during four days at 40° C. (104° F.) too small to be accurately measured, except with one sample of stored centralized butter.

---

[13] Jr. Dairy Sc. 5. P. 51. 1922.

[14] See ref. 13.

[15] Jr. Dairy Sc. 5. P. 64. 1922.

[16] U. S. D. A. B. A. I. Bul. 57. 1904.

In the investigations of Rogers[18] the action of milk lipase was materially weakened by an exposure of 10 minutes at 45° C. (113° F.) and was entirely destroyed at 60° C. (140° F.) for this period. Rogers, Berg and Davis[19] found that with the continuous method of pasteurization lipase was destroyed at 70° C. (158° F.).

The hydrolysis of fat would be an objectionable change in certain dairy products, especially since some of the fatty acids that might be set free have very disagreeable odors. In butter, with its high content of fat, the possibility of fat decomposition being a factor in deterioration should be taken into consideration. An increase in the acidity of this product is sometimes observed on holding and, while this may be due to bacterial changes, the activity of lipase must also be recognized as a possible cause. Rice and Markley[20] found some evidence that the rancidity of sweetened condensed milk is due to milk lipase.

The abnormal flavor of milk that is sometimes noted with advanced lactation appears to be due to lipase in unusually large amounts.[21]

Catalase. The enzyme catalase which splits hydrogen peroxide into water and molecular oxygen according to the reaction $2H_2O_2 = 2H_2O + O_2$ is regularly found in milk. Its presence can be shown by adding a small amount of hydrogen peroxide to milk and observing the liberation of bubbles of oxygen. The use of a definite volume of milk with a standard amount of hydrogen peroxide and some sort of a tube in which the gas liberated can be collected and measured, permits a comparison of the activity of the catalase in various lots of milk. Certain micro-organisms produce catalase so that their action may be a factor in the results obtained. Excessive numbers of body cells also seem to increase the catalase activity of milk.

Catalase is widely distributed in nature and is present in the milk of a variety of animals. The catalase contents of various lots of milk differ considerably, with colostrum milk and that from cows advanced in lactation containing especially large amounts. Cream and separator slime contain more catalase than milk. Thatcher and

[17] See ref. 7.

[18] See ref. 16.

[19] See ref. 11.

[20] See ref. 15.

[21] Jr. Dairy Sc. 5. P. 201. 1922.

Dahlberg[22] examined a number of samples of butter and noted that each contained catalase; storage did not appear to decrease its activity.

Rogers, Berg and Davis[23] found catalase was destroyed in cream pasteurized by the continuous method at 70° C. (158° F.).

Catalase is apparently of little importance in milk or its derivatives. Since excessive amounts are sometimes due to large numbers of bacteria or leucocytes or some other objectionable condition, a determination of the catalase activity of milk has been suggested as a test for the sanitary quality. Such a determination has also been proposed as a test for pasteurization; although catalase is not completely destroyed until rather high temperatures are reached, it is influenced at temperatures considerably lower.

Peroxidase. Milk normally contains a peroxidase which is an enzyme that oxidizes readily oxidizable substances by transferring oxygen to them from some peroxide, for example, hydrogen peroxide. It may be demonstrated in milk or cream by adding to a small volume a few drops of hydrogen peroxide and some indicator which is readily oxidized and which, when it is changed, gives a pronounced color so that detection of the oxidation is easy. Satisfactory indicators are freshly prepared 10 per cent gum guaiac in alcohol, 1.5 per cent aqueous solution of ortol, 2 per cent aqueous solution of paraphenylenediamine, etc.; a large number of indicators have been employed, some of which are mixtures of reagents. The indicator is often added before the hydrogen peroxide so that oxidases can be detected. Peroxidase seems to be more active in cream than in milk.

Rogers, Berg and Davis[24] found that in cream pasteurized with the continuous method peroxidase was absent after heating to 79° C. (174.2° F.) or above; at 77° C. (170.6° F.) it was generally absent while at 74° C. (165.2° F.) or below it was always present. Spitzer and Taylor[25] reported that 62.5° C. (144.5° F.) for 20 minutes had no apparent effect on the peroxidase in milk while flash pasteurization at 85° C. (185° F.) inactivated the greater part of it.

The peroxidase of milk affords a basis for determining whether or not milk and its various derivatives have been heated to a comparatively high temperature. This is important in enforcing certain pasteurization requirements, especially those dealing with the pasteurization of factory by-products, but cannot be used to show whether or not milk has been heated to the exposures usually employed with market milk because the temperature required to destroy peroxidase is too high. It seems probable that, in certain instances, the pasteurization requirements intended to prevent the spread of animal diseases through skim milk, etc., were selected with the idea of the presence of peroxidase providing a means of detecting violations. The peroxidase test has also been employed with butter to secure information on the heat to which the cream was subjected; the method usually followed consists of melting the butter, recovering the water with the dissolved and suspended material, by pipetting or using a separatory funnel, and then adding the reagents to this. Peroxidase in butter seems, in certain instances, to disappear during holding. Rogers, Berg and Davis[26] found that in buttermilk the enzyme disappeared in about half of their samples during a holding period of one year and ascribed this to the slow digestion taking place. These investigators state that the peroxidase reaction in raw milk can be made to disappear in about 20 or 25 days, by allowing the milk to sour, adding chloroform to eliminate subsequent bacterial action and then allowing proteolysis by the lactic acid to go on, either with or without the aid of pepsin; they also point out that salts of iron may be responsible for a reaction indistinguishable from a peroxidase reaction.

---

[22] See ref. 7.

[23] See ref. 11.

[24] See ref. 11.

[25] Jr. Dairy Sc. 7. P. 234. 1924.

Dahle and Palmer[27] investigated the effect of storage on the peroxidase activity of whole milk powders and concluded that factors known to favor oxidation, such as air, heat and moisture, proved detrimental to peroxidase activity. Powders stored in a vacuum or in containers which prevented the entrance of air and moisture showed greater peroxidase activity than the same powders exposed to air and moisture.

There is the possibility that peroxidase, because of its oxidizing properties in the presence of a peroxide, may be a factor in causing changes in various dairy products. The deterioration of butter is known to involve oxidations and peroxidase is one of the possible factors responsible for this. However, Palmer and Miller[28] found that the addition of peroxidase concentrated from horse-radish roots to pasteurized cream had no effect on the keeping quality of the butter churned from it, either with or without ripening of the cream; some of the butter was stored at room temperature and some at 0° C. (32° F.).

[26] See ref. 11.

[27] Jr. Dairy Sc. 7. P. 141. 1924.

[28] Jr. Dairy Sc. 9. P. 272. 1926.

CHAPTER X

BACTERIOLOGY OF EVAPORATED, SWEETENED CONDENSED AND POWDERED MILK

Large quantities of milk are manufactured into evaporated, sweetened condensed and powdered milks for the purpose of supplying persons in areas where fresh milk is not readily available and also to provide milk that can be stored in the home on the same basis as many other food products. When properly manufactured and stored these materials have good keeping qualities, but with evaporated and sweetened condensed milks abnormal conditions sometimes develop as a result of the action of micro-organisms. In general, the organisms causing these abnormalities are types that are not important in normal milk and cream and, moreover, the ones growing in evaporated milk are distinctly different than those developing in sweetened condensed milk because of differences in the methods of manufacture, especially the addition of sucrose in the sweetened milk. Accordingly, evaporated and sweetened condensed milks each present a more or less characteristic problem in so far as micro-organisms are concerned.

BACTERIOLOGY OF EVAPORATED MILK

The keeping property of evaporated milk depends on the destruction of the contained organisms by the heating to which the sealed cans are subjected, either in an autoclave or in a continuous sterilizer. The nutrients and water present make evaporated milk well suited to the growth of many types of bacteria and, since nothing is added to control the development of micro-organisms, changes commonly take place if the sterilization is incomplete. In some instances bacteria are present without the evaporated milk appearing abnormal; these types apparently grow very slowly, if at all, in this product.

Sterilization exposures with evaporated milk. The proper sterilization exposure with evaporated milk is very important. The heating must not be too severe because of the undesirable changes likely to result and it must be sufficient to cause the destruction of all the bacteria contained. No general statement can be made as to the exposure required since it varies with a number of factors, and exposures that are entirely satisfactory under one set of conditions may not be under another. Because of the development of some unusual situation, condenseries sometimes find it necessary to change from an exposure that has been satisfactory over long periods; most often the exposure must be increased to destroy some very resistant organism that has suddenly begun to cause trouble.

The sterilization exposures used in many plants vary from 115.6° C. (240° F.) to 118.3° C. (245° F.) for 15 or more minutes. In addition, there is an exposure to heat during the time the sterilization temperature is being reached and often this temperature increase is carried out at a definite rate. The exposures used are commonly varied slightly with the different sizes of cans and with the quality of the milk. Certain operators have various minor procedures that they consider are important in carrying out the sterilization process.

Incubation of evaporated milk. After sterilization the cans are often incubated at the plant for from one to several weeks with the idea of giving the leaky and incompletely sterilized cans a chance to spoil so that they can be easily detected. In some instances this incubation is carried out at ordinary plant temperatures, while in others a special room that is kept warm is used because the organisms most often causing spoilage are greatly favored by comparatively high temperatures. The incubation usually accomplishes its purpose and the defective cans are readily picked out, either by the appearance or by shaking. Occasionally, however, the organisms that have not been killed by the heating develop slowly and do not bring about changes until the cans have been held for extended periods; this is especially serious when the spoilage is not evident until the cans are in the hands of the retailer or consumer.

Types of spoilage due to organisms. The fermentations that improperly prepared evaporated milk undergo are of various types, the more common being coagulation, gas formation, and the development of off-flavors, especially bitterness. The responsible organisms either resist the heat exposures used or get in after the heating through defects in the cans.

Coagulation of evaporated milk. The coagulation of evaporated milk may be brought about by various organisms. It is most commonly due to spore forming types that induce a typical sweet curdling but occasionally occurs as a result of the activity of the ordinary lactic acid bacteria. Since these latter organisms can not be expected to survive any of the exposures that would be tried for the sterilization of evaporated milk, their presence must be due to tiny defects in the cans. This explains why coagulation due to the lactic acid bacteria involves only an occasional can and never any considerable portion of a batch.

The Vermont Agricultural Experiment Station[1] reported an instance of the coagulation of evaporated milk in which the change was noted in from 12 to 24 hours after heating; the milk curdled without souring and then the curd apparently digested, leaving a whey-like liquid above a mass of slimy curd. *B. subtilis* was the organism most actively involved in the abnormal condition.

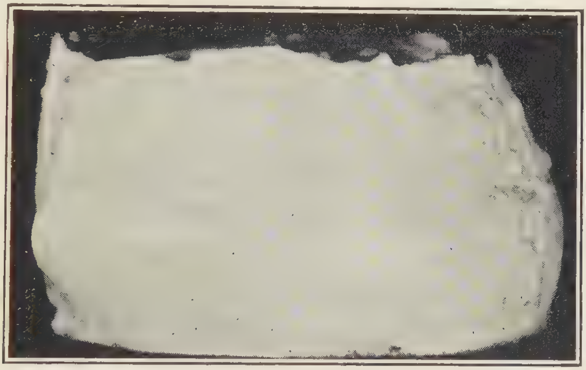


Fig. 24.—Material from a can of evaporated milk artificially inoculated with *Bacillus coagulans.* Iowa Agr. Expt. Sta. Res. Bul. 19.

Hammer[2] studied an outbreak of abnormal evaporated milk in which the typical change was a firm coagulation, although a very small percentage of the spoiled cans that were not included in the investigation showed a bulging. It was reasonably certain that at least some of the spoiled cans received an exposure of 113.3° C. (236° F.) for 36 minutes. The May and June milk gave the greatest percentage of spoilage. It was impossible to state the time required for coagulation but the heated milk was kept at the plant for at least ten days and had not coagulated after this holding period. The spoiled milk had a sweetish, cheesy odor, not at all disagreeable, and a flavor that was faintly sour and cheesy. The acidity was higher than that of unspoiled cans from the same batch. An organism was quite regularly observed in microscopic preparations from the abnormal milk and, when it was cultivated and inoculated into normal cans of evaporated milk, it produced the typical abnormal condition. The organism, which seemed to be a species not previously described, was named *Bacillus coagulans.* Cordes[3] investigated an outbreak of "flat-sours" in a condensery and found that *B. coagulans* was responsible. The isolated organism was studied for its heat resistance by subjecting freshly inoculated milk cultures to steam under pressure in an autoclave, the temperature being determined by a maximum registering thermometer placed in a tube of milk. Under these conditions the organism survived 106.1° C. (223° F.) for two minutes and also a maximum of 107.2° C. (225° F.), even when there was considerable additional exposure to heat due to the time required to reach these temperatures. It was killed, however, by a temperature of 112.2° C. (234° F.) when six minutes elapsed in heating from 100° C. (212° F.) to 112.2° C.

---

[1] Vt. Agr. Expt. Sta. Bul. 170. 1912.

[2] Ia. Agr. Expt. Sta. Res. Bul. 19. 1915.

Kelly[4] studied three types of spoilage in evaporated milk. In type A, a custard-like clot was formed on top of the milk but there was no other change, in type B all of the milk in the can clotted, with a clean even curd and a slightly bitter, metallic flavor, while in type C some gas was formed and the milk was clotted solid and had a cheesy odor. The responsible organisms were considered to be an atypical form of *Bacillus cereus* for type A, an atypical form of *Bacillus simplex* for type B and an atypical form of *Bacillus megatherium* for type C.

Heat coagulation of evaporated milk. During the sterilization of evaporated milk an incipient coagulation is desirable since, when the coagulum formed in this way is broken up by the shaking process used, it produces a creamy body in the product. Milk varies a great deal in the rate of coagulation with heat, the coagulation sometimes being too slow and sometimes too rapid. Sommer[5] concluded from his consideration of heat coagulation that the main factor is the salt composition of the milk. There is a balance between the calcium and magnesium and the citrate and phosphate and an excess of either class of salts causes coagulation, while a proper balance produces the most stable condition. In evaporated milk, when the coagulation was due to an unsatisfactory salt balance, an excess of

[3] Jr. Dairy Sc. 11. P. 46. 1928.

[4] Trans. Royal Soc. Canada. Section 5. P. 387. 1926.

[5] Proc. World's Dairy Cong. 2. P. 1241. 1923.

calcium and magnesium was found in all cases so that the addition of sodium citrate or di-sodium phosphate was required to improve the milk. This investigator lists the other factors in the heat coagulation of evaporated milk in the following order of importance: albumin content, acidity and rennin forming bacteria. Micro-organisms appear to be of very little importance in this connection.

Gas formation in evaporated milk. Cans of evaporated milk may undergo a gassy fermentation which results in a bulging, particularly at the ends of the cans, and sometimes in a bursting along the seam. Usually coagulation accompanies the gas formation and often there is the development of objectionable odors. When bulged but unbroken cans are punctured, the contents may foam through the opening for considerable periods. Because of variations in the condition of the contents of the gassy or ''blown'' cans, it is evident that the causative organism is not always the same and, in all probability, mixtures of organisms are sometimes responsible for the changes observed.

The spore forming anaerobes constitute one of the important groups responsible for gas formation. These organisms are unusually resistant to heat and, accordingly, may survive the heat exposures used; they sometimes cause numbers of ''blown'' cans in certain batches of evaporated milk. Various species seem to be involved and while some of them develop considerable acid, especially butyric, others produce very objectionable putrefactive changes. The presence of spore forming anaerobes in milk intended for evaporation is to be expected because of their presence in the milk coming from the ordinary farms.

Non-spore forming organisms have also been noted as the cause of gas development in evaporated milk. Savage and Hunwicke[6] isolated various species belonging to the Escherichia-Aerobacter group as well as coccus forms.

Hammer[7] secured a Streptococcus, which he designated *Streptococcus distendens*, from a can of evaporated milk showing slight gas formation. In a considerable number of inoculation experiments this organism never produced sufficient gas to burst a can; although it grew well in ordinary sterile milk, gas formation was not noted unless the milk was sealed in some way, for example, with a mixture of vaseline and paraffine. *S. distendens* was isolated from evaporated milk in which other non-spore forming organisms were also growing so that it seems probable the organisms gained entrance after the heating.

---

[6] Food Insp. Bd. London. Spec. Rpt. 13. 1923.

[7] Ia. State Col. Jr. Sc. 2. P. 1. 1927.

It should be noted that in a can of evaporated milk a slight gas development, which might go unnoticed in a tube of milk, makes itself evident because all of the gas produced is held. Inoculation into a can of milk thus affords a convenient method of detecting slight gas formation with organisms that will grow in this material.

The bulging of cans of evaporated milk may be due to causes other than bacterial action. The more common of these are as follows:

1. Bulging due to chemical action. The acid present in evaporated milk may act on the metal and combine with a considerable part of the iron, thus liberating hydrogen. This is not likely to occur except after long periods. The action has been demonstrated experimentally by Hunziker and Wright.[8] Tin cans were scratched on the inside with a file, filled with a 0.4 per cent solution of lactic or acetic acid and then sealed and sterilized; on incubating at 32.2° C. (90° F.) the cans began to swell while the check cans containing distilled water did not.

2. Bulging due to filling cans with very cold milk. When cans are filled with very cold milk and sealed, they may, after processing, show a bulging at ordinary temperatures due to the expansion of the contents. Variations in atmospheric pressure may be a contributing factor in this type of bulging.

It is ordinarily easy to recognize bulging that is due to bacterial action by the odor and other changes in addition to the gas formation and by the presence of considerable numbers of bacterial cells in microscopic preparations.

Bitter evaporated milk. A bitter flavor in evaporated milk is occasionally encountered. Sometimes it accompanies coagulation, as would be expected from the fact that most organisms sweet curdling milk also digest it with the formation of bitter products, while in other cases it appears to be the conspicuous change and coagulation goes unnoted.

Hammer[9] investigated an instance of bitterness in evaporated milk and isolated an organism which produced the condition on inoculation. The first isolation was made from a can showing bitterness and an abnormal odor but no evident coagulation, while later the same organism was secured from a can of coagulated milk from the plant reporting the trouble. With extended holding periods, inoculated evaporated milk showed complete coagulation; microscopic examinations of this milk suggested that the organism had largely died out. The organism was named *Bacillus amarus.*

---

[8] Hunziker, O. F. Condensed Milk and Milk Powder. 4th ed. P. 419. 1926.

[9] Ia. Agr. Expt. Sta. Res. Bul. 52. 1919.

Spitzer and Epple[10] studied bitter evaporated milk in which the bitterness was the only objectionable feature. The condition did not appear in all of the cans of a batch. The causative organism agreed morphologically and culturally with *Bacillus panis.* It rapidly produced large quantities of peptone and lower complex nitrogenous compounds which may have been the cause of the bitterness. The organism was found to be destroyed at an exposure between 8 and 10 minutes at 121.1° C. (250° F.) under steam pressure.

Fishiness in evaporated milk. A can of evaporated milk which showed a fishy flavor was investigated by Hammer[11] and an organism capable of producing fishiness in milk, cream and evaporated milk isolated. In inoculated milk there was, in addition to the development of a fishy flavor, a coagulation and rapid digestion. The organism was named *Bacillus ichthyosmius* and its close relationship to the Proteus group recognized.

Bulk evaporated milk. Bulk evaporated or condensed or superheated condensed milk is extensively used in the ice cream industry in raising the milk solids content of the ice cream mix. This material has limited keeping qualities because it is not sterilized and nothing is added to control the growth of micro-organisms. Often it is held at condenseries for some time and then shipped without refrigeration. The most common change noted in this type of milk is the development of acid but other fermentations also occur.

Ruehe[12] concluded from his investigations that with bulk evaporated milk the manufacturing process is very efficient in reducing the bacterial content of the raw milk. The maximum destruction of organisms in a number of batches was 99.999 per cent, the minimum 99.874 and the average 99.969. The maximum bacterial content of the evaporated milk was 36,400 per cc., the minimum 480, and the average 6,289. The changes in the numbers of bacteria during the making process are illustrated by the following data representing nine batches:

---

[10] Jr. Dairy Sc. 3. P. 486. 1920.

[11] Ia. Agr. Expt. Sta. Res. Bul. 38. 1917.

[12] N. Y. (Cornell) Agr. Expt. Sta. Mem. 76. 1924.

Changes in the Numbers of Bacteria during the Making of Bulk Evaporated Milk

	Bacteria per CC.				
	Raw Milk	After Heating to 65.6° C. (150° F.)	Held 10 Minutes at 65.6° C.	After Concentrating	After Superheating
Maximum..	136,000,000	360,000	70,500	145,000	36,400
Minimum..	1,150,000	17,500	7,800	2,260	1,020
Average....	52,209,000	146,200	31,900	27,100	6,548

When the condensing equipment was not properly cleaned and sterilized it proved to be a serious source of contamination. As a result of his studies, Ruehe considers that the bacterial content of bulk evaporated milk should be relatively low and that the high counts often noted as the material is used in ice cream plants is due to contamination or growth after the manufacturing process.

BACTERIOLOGY OF SWEETENED CONDENSED MILK

The keeping quality of sweetened condensed milk depends on the concentration of the milk solids and added sucrose, although the destruction of organisms during the condensing is probably also a factor. In analyses of various brands compiled by Hunziker [13] the milk solids varied from 29.40 to 36.57 per cent, the water from 21.67 to 32.15 per cent and the sucrose from 37.55 to 43.42 per cent. The plasmolyzing action of the materials in solution effectively prevents the action of most organisms. This is shown by the spoiling of diluted sweetened condensed milk under conditions with which the undiluted product shows no change. Sweetened condensed milk keeps for considerable periods when it is allowed to stand open at temperatures satisfactory for the growth of many micro-organisms.

Rice and Downs [14] have investigated the importance of the sugar concentration in the keeping quality of sweetened condensed milk. The percentage of sugar in solution in the water was calculated from the percentages of sugar and water and was designated the sugar ratio; for example, a sample with a sucrose content of 46.6 per cent

[13] Hunziker, O. F. Condensed Milk and Milk Powder. 4th ed. P. 290. 1926.

[14] Jr. Dairy Sc. 6. P. 532. 1923.

and a moisture content of 27.4 has a sugar ratio of 63.0. It was found that in order to surely prevent growth of the organisms causing thickening in sweetened condensed milk it was necessary that the sugar ratio be at least as high as 64.5. As the ratio fell below this the organisms became more and more active until at 63.0 a strong odor developed and actual solidification began.

The organism isolated by Hammer[15] from gassy sweetened condensed milk grew in a saturated sucrose bouillon but failed to produce gas in one brand of sweetened condensed milk into which it was inoculated. This suggests that the milk solids present may be a factor in restraining growth as well as the sucrose, especially since the brand in which the organism would not grow was one high in milk solids.

Organisms in normal sweetened condensed milk. A number of investigators have noted that sweetened condensed milk lacks sterility and a few have reported the numbers of organisms found, which vary from something under 100 to 100,000 per cc. It is probable that the quality of the materials employed and the care used in handling are important factors in determining the numbers. Cocci of various types have probably been the most common organisms reported but a number of others have also been isolated. Downs[16] stated that the bacterial content, determined by the plate method, of sweetened condensed milk shortly after its manufacture is a good indication of the sanitary conditions of the plant producing it.

Coutts[17] has considered the growth of bacteria in sweetened condensed milk. He referred to the work of Sandilands, who found only slight increases, and to the work of Heggs, who noted small increases with most brands but an increase from 21,800 to 114,700 per cc. in three days at 22° C. (71.6° F.) with one brand. Andrewes[18] found that *Staphylococcus aureus* increased greatly and his experiments suggest that condensed milk is almost a differential medium for the growth of staphylococci.

It, accordingly, seems that sweetened condensed milk contains numbers of organisms and that these may undergo some growth and the product still remain normal. With certain conditions, the growth of micro-organisms may be very rapid but the types involved must be capable of tolerating high concentrations of materials in solution.

---

[15] Ia. Agr. Expt. Sta. Res. Bul. 54. 1919.

[16] Jr. Dairy Sc. 8. P. 344. 1925.

[17] Rept. Local Govt. Bd. on Pub. Health and Med. Subj. Food Rpt. 15. 1911.

[18] Jr. Path. and Bact. 18. P. 169. 1913.

Types of spoilage due to organisms. The more frequent changes which sweetened condensed milk undergoes as a result of the activity of micro-organisms are gas formation, thickening and the development of buttons.

Gas formation in sweetened condensed milk. The development of gas in sweetened condensed milk often results in the bursting of the cans along the seams. In certain instances this fermentation is very serious because it may appear suddenly and involve practically the entire pack for a number of days before it is detected. When most of the cans in a carload blow up, the loss in money and reputation of the brand is very considerable.

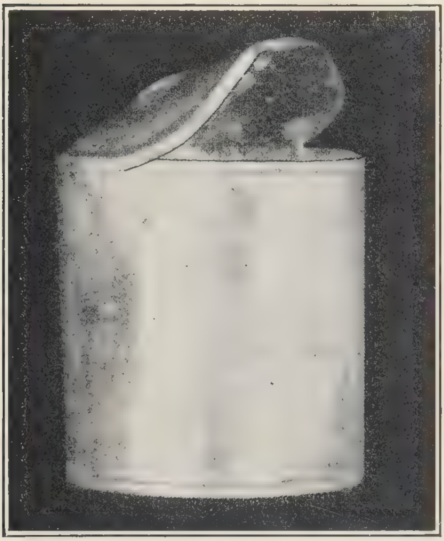


FIG. 25.—Can of sweetened condensed milk blown open after artificial inoculation with *Torula lactis-condensi*. Iowa Agri. Expt. Sta. Res. Bul. 54.

The organisms usually responsible for the development of gas in sweetened condensed milk are torulæ. These organisms have been reported by Pethybridge,[19] Hunziker,[20] Hammer,[21] Knudsen[22] and others. Hammer named the organism studied by him *Torula lactis-condensi*. It was isolated from a very serious outbreak of blown milk involving about ten batches put out at one plant; seven to eight days elapsed between the canning of the milk and the development of pronounced swelling. The trouble largely, but not entirely, involved milk that had passed through a certain filler in which it was more exposed than in the other filler used in the plant. The development of gas was so extensive that the cans were greatly bulged and often ruptured along the seams. When swollen but unbroken cans were punctured, the milk and large volumes of gas foamed through the opening for a considerable time. The fermented milk had a yeasty flavor and aroma but was not disagreeable and there was no suggestion of putrefaction. A yeast was evident in microscopic preparations and was readily isolated by plating out the material on beef extract agar to which 30 per cent sucrose had been added. Inoculation trials with one brand of sweetened condensed milk gave only negative results while with another brand gas formation was usually secured. The organism grew well in bouillon to which 30 per cent sucrose had been added and produced considerable quantities of alcohol in this medium; it also grew in bouillon saturated with sucrose. Results were secured which indicate that yeasts capable of growth in 30 per cent sucrose bouillon may be rather commonly isolated but that most of these cannot produce gas in sweetened condensed milk.

---

[19] Econ. Proc. Royal Dublin Soc. 1. P. 306. 1906.

[20] Hunziker, O. F. Condensed Milk and Milk Powder. 4th ed. P. 388. 1926.

[21] See ref. 15.

[22] Den. Kgl. Veterinaer-og Landbohojskole Aarsskrift. P. 282. 1922.

Rogers and Clemmer[23] found an organism belonging to the Escherichia-Aerobacter group producing gas in sweetened condensed milk containing from 40 to 45 per cent sucrose. The temperature of the milk in the forewarmer was high enough to destroy the organism but an inspection of the plant experiencing the trouble showed abundant opportunity for infection, particularly by flies, between the pan and the can filler.

The data available show that the organisms generally responsible for gas formation in sweetened condensed milk are not resistant enough to survive the heating employed in the manufacturing process so that contamination after the condensing must be the cause of the abnormality. The contamination probably most often comes from the plant equipment.

Thickening of sweetened condensed milk. Sweetened condensed milk sometimes undergoes a thickening. In the early stages only portions of the milk in a can may be involved while later the milk may be thick throughout and pour only with difficulty. The thickening is sometimes the result of a physical change induced by high storage temperatures or long holding periods but may also be due to the action of micro-organisms. With continued growth of the organisms causing thickening, there is usually the development of an abnormal flavor and aroma.

Rice and Downs[24] studied the bacterial thickening of sweetened condensed milk and found it due to a coccus, which is capable of inverting and destroying sucrose and producing considerable acid. It grows in fairly high sucrose concentrations but can be checked

[23] Absts. Bact. 2. P. 6. 1918.

[24] See ref. 14.

if the concentration is sufficiently high; at temperatures above normal room temperature it is particularly active. This organism was isolated from factory equipment which suggests that the contamination of the milk may come from this source.

Downs[25] reported that the organisms thickening sweetened condensed milk belonged to one general type of Micrococcus of which two distinct varieties were noted. Many organisms of common occurrence closely resembled these and could only be told from them by their effect on sweetened condensed milk. This investigator believes that the thickening is due to the production of a rennin-like enzyme by the responsible organisms; the non-importance of acid is shown by the failure of certain organisms producing a high acidity in the milk to thicken it and also by the sweet curdling of litmus milk in which the organisms causing thickening had grown. An exposure of 64° C. (147.2° F.) for 10 minutes killed the organisms in broth.

Buttons on sweetened condensed milk. Small button-like masses of variable size are occasionally present on the surface of sweetened condensed milk and these are referred to as buttons. They are not firmly adherent to the milk and can be readily lifted off. Ordinarily they develop comparatively slowly.

Rogers, Dahlberg and Evans[26] found that buttons are due to the growth of *Aspergillus repens* and possibly other molds and that the using up of the air in a can prevents the continued development of the mold; they suggest that the button itself is probably due to enzymatic action which is continued after the death of the mold. The development of buttons may be prevented by keeping out the molds through careful methods or by making the conditions unsatisfactory for their growth, either by the use of a low holding temperature or by excluding oxygen. These investigators noted that buttons did not develop in milk held at 20° C. (68° F.) and that sealing the cans under a vacuum of 20 inches or more prevented their formation.

Knudsen[27] reported that in Europe buttons in sweetened condensed milk could often be traced to *Aspergillus glaucus;* another mold, *Catenularia fuliginea,* was found which forms somewhat similar buttons.

Enzymatic and chemical action in the spoilage of sweetened condensed milk. Both enzymatic and chemical action seem to be involved in the deterioration of sweetened condensed milk. The results of Rice and Markley[28] indicated that rancidity is due to the

[25] See ref. 16.

[26] Jr. Dairy Sc. 3. P. 122. 1920.

[27] See ref. 22.

[28] Jr. Dairy Sc. 5. P. 64. 1922.

action of the lipase of milk and Rice[29] has reported data supporting this conclusion. The enzyme hydrolyzes the milk fat and thus forms fatty acids, to some of which the rancid flavor is due. Rice found that the addition of only 0.75 per cent of unheated milk to a batch of sweetened condensed milk may cause rancidity; this was equivalent to 0.3 per cent of the amount of whole milk from which the batch was prepared. The addition of pancreatic lipase produced the same result but the condition was not produced by the organisms tried. Heating whole milk to 60° C. (140° F.) for 15 minutes or 65.6° C. (150° F.) for an instant destroyed the lipolytic power but when sugar was present or when condensed milk was heated a higher exposure was required. Rice considers that leaky intake valves and carelessness in heating the milk in the hot wells are the factors that may result in the presence of active lipase in the finished milk.

Tallowiness in sweetened condensed milk has been investigated by Rice,[30] who has pointed out that it is due to the action of the oxygen of the air on the butter fat, the reaction ordinarily being catalyzed by small amounts of copper. Biological agencies were excluded as a cause of tallowiness because (1) the condition apparently developed at 0° C. (32° F.) as rapidly as at room temperature, (2) neither heat sterilization nor strong preservatives prevented it and (3) the bacterial counts on tallowy samples were low. Tin did not catalyze the reaction while iron did to a very much less degree than copper.

BACTERIOLOGY OF MILK POWDER

When properly stored, milk powder does not undergo changes due to the action of micro-organisms because of the low moisture content. This product, however, contains organisms of various types, some of which have survived the drying while others are the result of subsequent contamination.

Methods of determining the bacteria in milk powder. The number of bacteria per gram of milk powder may be determined by weighing out a sample on sterile paper, adding this to sterile water in a flask, shaking until the milk is thoroughly distributed and then plating in the usual way. One gram of milk powder in 99 cc. of water is a convenient ratio. Supplee and Ashbaugh[31] used a microscopic method, the powder first being mixed with water in a proportion which gave the concentration of milk solids normally found in milk.

---

[29] Jr. Dairy Sc. 9. P. 293. 1926.

[30] Jr. Dairy Sc. 9. P. 459. 1926.

[31] Jr. Dairy Sc. 5. P. 570. 1922.

Organisms in milk powder. Downs [32] secured results on the bacterial content of milk powders that may be summarized as follows:

BACTERIA IN MILK POWDERS

Number of Samples	Method of Drying	Bacteria per Gram		
		Maximum	Minimum	Average
10	Single roll, milk previously condensed	626,000	16,900	49,500
10	Spray, milk previously pasteurized and condensed	595,000	15,600	178,000
9	Spray, milk not previously condensed	3,000,000	1,500,000	2,269,000

These data show that milk powders prepared by various methods regularly contained considerable numbers of organisms and that there were large variations in the counts.

Supplee and Ashbaugh [33] determined the bacterial content of fresh milk powders made in different factories by the Just double roller process and have presented the following data:

BACTERIA IN MILK POWDERS PREPARED BY THE JUST DOUBLE ROLLER PROCESS

Factory	Number of Samples	Bacteria per Gram		
		Maximum	Minimum	Average
1	10	10,000	700	4,600
2	26	170,000	2,000	27,300
3	34	96,000	700	18,800
4	13	37,000	800	5,000
5	28	6,360	350	1,500
6	22	8,600	2,100	3,400
7	25	1,400	400	790

Wide variations in the averages for the different plants and also in the different counts on samples from any one plant are evident from these results.

The comparative bacterial content of liquid milk and the powder made from it has been investigated by Supplee and Ashbaugh;[34] representative data from their studies are as follows:

---

[32] Thesis, Cornell Univ. Data given by Supplee and Ashbaugh. See ref. 33.

[33] Jr. Dairy Sc. 5. P. 216. 1922.

COMPARISON OF THE NUMBERS OF BACTERIA IN MILK AND IN THE POWDER MADE FROM IT

Sample Number	Bacteria per CC. in Liquid Milk	Bacteria per Gram in Powder from Cylinder
1	2,750,000	270
2	12,000,000	550
3	147,000,000	600
4	280,000,000	880
5	345,000,000	520
6	1,520,000	520
7	2,300,000	500

From these determinations it is evident that the numbers of bacteria surviving the drying process are low and that there is no very direct relationship between the numbers before and after drying. With milk dried by the Just process, Supplee and Ashbaugh[35] found that some of the organisms originally present are no longer detectable by the microscopic method. In reconstituted milk the microscopic method showed from 30 to 78 per cent of the organisms originally present in the natural milk, with an average of 45.3 per cent. A tendency for the groups of bacteria existing in natural milk to be broken up during the drying process was noted.

The contamination of milk powder under routine factory conditions has been studied by Supplee and Ashbaugh[36] and the following data are taken from their report:

CONTAMINATION OF MILK POWDER SUBSEQUENT TO DRYING

Sample Number	Bacteria per Gram		
	Milk Solids from Cylinder	After Sifting	After Packing
1	400	1,400	2,300
2	960	1,400	2,300
3	1,100	3,000	4,300
4	600	2,200	5,200

---

[34] See ref. 33.

[35] See ref. 31.

[36] See ref. 33.

The results show that, on a percentage basis, the contamination of the milk powder during its preparation for market is very considerable.

The bacteria found in milk powders are of various types and include especially cocci and spore formers. Yeasts and molds are also present.

Changes in numbers of organisms. Because the low moisture content makes conditions so unfavorable, a decrease in the bacterial content of milk powder during holding would be expected. Supplee and Ashbaugh [37] concluded that the bacteria in milk powder die off rapidly during storage and that, in normal powders made by the Just process, the numbers reach an approximate constancy after two to four months. Milk powders to which organisms were added showed a similar decrease but a longer period was required to reach an approximately constant number.

Effect of drying on various organisms. The effect of the drying process on various organisms has been studied by Hunwicke and Jephcott [38] using an experimental drying plant identical with the factory machines except that the rollers were reduced. They found the non-spore forming bacteria were completely destroyed while bacterial spores were not destroyed in all cases, the number of survivors apparently being dependent on the extent to which sporulation has occurred. Dried milk prepared from milk highly infected with *M. tuberculosis* was incapable of causing lesions in guinea pigs.

Deterioration of milk powders. The decrease in numbers of bacteria in milk powders is of interest in indicating that the deterioration of this product is due to causes other than the action of bacteria. Supplee and Ashbaugh [39] concluded that, "The presence of large numbers of bacteria in desiccated milk does not produce any detectable effect upon the keeping quality in the presence of moisture concentrations which would admit the powder as a commercial article."

Supplee [40] has pointed out that deterioration in skim milk powder involves the development of a stale condition evident as a characteristic taste and odor, a decreased solubility of the casein and frequently a darkening of the color. Moisture is an active factor in hastening the development of these conditions and it may be that moisture originally left in the product or that absorbed from the air. This indicates the necessity of protecting milk powders so that moisture will not be taken up. Whole milk powder is subject to the same deterioration as skim milk powder and additional changes involving the fat, such as the development of rancidity and tallowiness. Supplee reported that lipase artificially introduced into Just process powder of low moisture content caused the development of pronounced rancidity within a few weeks while the check samples were not so affected but developed tallowiness after a long time. This investigator points out that the effect of lipase is significant since enzymes are not usually destroyed by the spray methods in common use while they are inactive in powders made by the Just process. Supplee also noted that in powder which had developed tallowiness there is commonly a decrease in the iodine number of the fat. Comparatively high temperatures and the presence of metallic catalyzers, especially copper or copper salts, hastened the development of tallowiness, while the holding of the powder in vacuum, nitrogen or carbon dioxide delayed it.

---

[37] See ref. 33.

[38] Jr. Dairy Sc. 8. P. 206. 1925.

[39] See ref. 33.

[40] Proc. World's Dairy Cong. 2. P. 1248. 1923.

Dahle and Palmer[41] also noted that whole milk powders with which the moisture content was increased by exposure to moist air exhibited very inferior keeping qualities and that the storage temperature was an important factor. There was little difference between 4° C. (39.2° F.) and 20° C. (68° F.) but at 37° C. (98.6° F.) deterioration was very rapid and the powders became hard and discolored. At 37° C. all the powders showed pronounced deterioration when first examined after three months' storage. In containers which completely excluded outside air and moisture there was almost no deterioration after one year at 4° C. or 20° C.

[41] Jr. Dairy Sc. 7. P. 40. 1924.

CHAPTER XI

BACTERIOLOGY OF ICE CREAM

The dissemination of pathogenic organisms through milk and cream and the importance of these materials in the manufacture of ice cream have shown the necessity of careful methods in the making and handling of this product. Bacterial counts have come into use as an aid in controlling these methods in the same way that they are used in supervising milk supplies and furnish valuable information with reference to the sanitary quality of ice cream. Although most of the bacteria in ice cream, as in milk, are entirely harmless the conditions responsible for large total numbers are the conditions most likely to result in the presence of harmful types or objectionable products of growth.

The bacteriological principles involved in the manufacture of ice cream are essentially the same as those involved in the handling of market milk since, with both products, bacteria are undesirable and should be kept out as completely as possible while those that do gain entrance should be destroyed or prevented from growing. However, the variety of raw materials used and the conditions of manufacturing and holding present problems not found in the market milk industry and these have received considerable attention in connection with attempts to develop satisfactory bacterial standards for ice cream.

Determining the number of bacteria in ice cream. The plate method is commonly used in determining the number of bacteria in ice cream. It is employed under the conditions that are standard for milk since the dairy products are ordinarily the most important source of the organisms present. Samples may conveniently be handled in sterile petri dishes or cotton stoppered flasks and should be taken with a sterile spoon or butter trier, the latter being especially convenient for sampling bulk ice cream. In all cases the material that was at the surface should be discarded; with a butter trier this is easily done by cutting the plug of ice cream over the edge of the lower half of a petri dish so that the portion of the plug from the surface falls outside the dish. Samples of ice cream should be melted rather rapidly, but without getting them above 40° C. (104° F.), and then thoroughly mixed. In pipetting the portion to be used in plating, which is usually one cubic centimeter, care must be taken that air bubbles are not included and this can best be done by slowly agitating the melted samples and then putting the tip of the pipette at the bottom of the layer of melted ice cream. At one time portions of about one gram were weighed out for use in plating but this method is now unusual because of the time required.

When ice cream improvers and powders containing rennin were used, the determination of the number of bacteria was sometimes complicated by the ice cream becoming a curdled mass when it was warmed. Such samples required weighing and even then the results were not entirely satisfactory since it was difficult to get a representative sample, due to the expression of the whey, and the material could not be uniformly distributed through the water blank.

The conditions that are standard for the plate method of determining the number of bacteria in milk give results with ice cream that are lower than might be secured with a different incubation. Ayers and Johnson[1] found that 30° C. (86° F.) for five days gave them practically double the count secured at 37° C. (98.6° F.) for 48 hours. In control work, however, the time required to secure the results is an important consideration and the shorter incubation period is to be preferred since the results are desired mainly for comparative purposes.

The direct microscopic method may be employed in determining the number of bacteria in ice cream but with the widespread use of pasteurization in the industry its value is somewhat limited.

Numbers of bacteria in ice cream. The bacterial content of ice cream is extremely variable due primarily to variations in the quality of the materials used and in the care taken in the manufacture and handling. Under very good conditions of production the counts may be as low as a few thousand or even under one thousand per cc. while with careless methods they may be well up in the millions. A count of 8,000,000,000 per cc. has been reported in one instance.[2]

There appears to have been a significant decrease in the bacterial content of ice cream within recent years. This has undoubtedly been the result of the attention given to the sanitary quality of this product in various cities and to the improvements that have been made in the methods of manufacture. The following table taken from the work of Fay and Martin[3] gives the low, high and median counts secured on the ice cream submitted to the annual ice cream scoring contests held at the Kansas State Agricultural College; it illustrates both the wide variations that occur in the bacterial content of different lots of ice cream and the improvement in the median count from year to year.

---

[1] U. S. D. A. Bul. 563. 1917.

[2] Milwaukee Bur. Econ. and Eff. Bul. 13. P. 35.

BACTERIAL CONTENT OF ICE CREAM SUBMITTED TO K.S.A.C. SCORING CONTESTS

	1921	1922	1923	1924	1925	1926	1927	Values for All
	28 Samples	39 Samples	48 Samples	53 Samples	42 Samples	35 Samples	46 Samples	291 Samples
Low count....	5,000	4,000	2,000	3,000	2,000	2,000	3,000	2,000
High count...	20,000,000	27,000,000	47,000,000	1,000,000	2,400,000	2,500,000	14,300,000	47,000,000
Median count.	200,000	87,000	80,000	70,000	53,000	46,000	32,000	66,000

Types of bacteria in ice cream. The bacteria present in ice cream are of a variety of types as would be expected from their sources. Ayers and Johnson[4] investigated the ice cream sold at retail in Washington and, by picking the colonies into litmus milk, found the various groups of bacteria in the following percentages:

GROUPS OF BACTERIA IN ICE CREAM SOLD AT RETAIL IN WASHINGTON

	Average Group Percentages in	
	71 Summer Samples	28 Winter Samples
Acid coagulating........	49.82	30.84
Acid non-coagulating....	20.72	38.03
Inert..................	13.98	4.81
Alkali formers..........	1.86	5.42
Peptonizers............	13.62	20.90

These results show that the acid forming organisms made up a considerable percentage of the total flora. Ellenberger[5] found these organisms predominating all through the storage period. From the standpoint of the growth of organisms, an ice cream mix is quite comparable to milk or cream and, if a mix or melted ice cream is allowed to stand at a temperature favorable for growth, the usual change is the development of acid by organisms of the *S. lactis* group.

---

[3] Jr. Dairy Sc. 11. P. 136. 1928.

[4] U. S. D. A. Bul. 303. 1915.

[5] N. Y. (Cornell) Agr. Expt. Sta. Mem. 18. 1919.

The occasional presence of pathogenic organisms in ice cream is evident from the instances in which this product has been responsible for outbreaks of disease.

SOURCES OF THE BACTERIA IN ICE CREAM

The bacteria in ice cream come from two main sources, (1) the materials used and (2) the contamination incident to the manufacture and handling.

Bacteria in the Materials Used

The materials used that require consideration from the standpoint of the bacteria in ice cream are the cream, the other milk derivatives, the gelatin, the sugar and the flavoring materials.

Cream. The cream employed in making ice cream commonly presents a real problem to the manufacturer. It is often shipped for considerable distances so that there is comparatively little direct control over the methods of production and handling. During the hot months, when the demand for ice cream is heavy, the cream production falls off and there may be difficulty in securing the desired quantities so that the ice cream manufacturer is often forced to be still less critical. In some sections the cream is shipped without refrigeration and this has a significant effect on bacterial growth. Because of this combination of conditions the cream coming to many plants contains large numbers of organisms.

Pasteurization enables the ice cream manufacturer to destroy a high perecentage of the organisms in the cream, including the pathogens that may be present. Both the numbers and percentages of the organisms destroyed depend on the types originally present and the exposure used but are regularly high with satisfactory methods. Pasteurization also gives the cream the keeping quality which is so necessary; the demand for ice cream varies with the weather and other factors and a supply planned for a number of warm days may involve a considerable surplus if there is a drop in temperature. The holding of properly pasteurized cream for reasonable periods is readily accomplished by the use of low temperatures, such as 0° C. (32° F.) or slightly lower.

Reconstituted cream presents the same general problems as normal cream. Each of the materials used in the reconstituting contains organisms in variable numbers, depending on the quality, but many of these are destroyed by the heat used.

Other milk derivatives. The milk derivatives other than the cream that are employed in the ice cream mix include especially bulk condensed milk and milk powder; the bacteriology of these has already been considered in Chapter X. It is necessary for the ice cream manufacturer using bulk condensed milk to recognize that there may be extensive bacterial development in this product, so that it requires careful cooling and storage. If a can of it is simply placed in a cooler the temperature falls very slowly; the center of the milk cools especially slowly because the viscosity prevents the development of convection currents that would aid in uniform cooling. In some instances bacterial growth in bulk condensed milk causes definite abnormalities, especially the development of acid.

Gelatin. The small percentage of gelatin used in an ice cream mix and the heat to which it is subjected in dissolving limit the influence of this ingredient on the bacterial content of ice cream.

A satisfactory procedure for determining the number of bacteria present in gelatin is to weigh out five grams on a sterile paper, transfer this sample to a 95 cc. water blank and then dissolve the gelatin by frequent shaking while the water is being heated to not over 40° C. (104° F.); this heating is readily accomplished by standing the water blank in tempered water. The gelatin solution is plated out, using the medium and general conditions employed with ice cream, and the counts calculated to a gram basis. The gelatin can also be weighed directly into the water blank.

The use of five grams of gelatin in 95 cc. of water is convenient from the standpoint of observing the turbidity and color of the gelatin solution. Moreover, when such a solution is hardened in a thin-walled container, shaking or jarring against the hand makes it possible to get some idea of the gelatinizing power of the product and while such ideas are not at all definite, the procedure affords a simple method of comparing two or more gelatins. By holding the hardened gelatin at a temperature which keeps it firm but which permits bacterial growth, a rough idea of the numbers of organisms in the gelatin can be secured since, under these conditions, colonies are formed; definite counts can not be made but the bacterial contents of two or more samples of gelatin can be compared and heavily contaminated samples easily detected.

Hammer[6] examined five samples of gelatin and found that the bacterial counts varied from 35 to 113,000,000 per gram. Parfitt[7] secured counts from 100 to 60,000,000 per gram on 17 samples. Brannon and Tracy[8] investigated 142 samples and found 12 free from organisms and 50 per cent containing less than 20,000 per gram; 31 per cent contained over 100,000 per gram. Some brands ran uniformly low in bacteria while others were much more heavily contaminated. Fay and Olson[9] reported counts on 50 samples; these averaged 1,636,000 per gram and varied from 10 to 108,000,000. Some of the results obtained at the Iowa Agricultural Experiment Station in the routine examination of lots of gelatin submitted from various sources are as follows:

---

[6] Ia. Agr. Expt. Sta. Bul. 134. 1912.

BACTERIAL CONTENT OF GELATIN

Source	Bacteria per Gram
A	1,300
B } different grades	65,600,000
B } different grades	940
B } different grades	300
C	135,000
D } different grades	11,700
D } different grades	2,240,000
E	7,000
F	166,000
G	10,000,000
H	600
I	242,000
J	12,000,000
K } different grades	24,400
K } different grades	10
K } different grades	26,200

The bacteria present in gelatin are of a variety of types. Spore formers are frequently encountered as are also cocci, some of which are chromogenic.

With the temperatures ordinarily used in dissolving gelatin large numbers of the contained organisms are destroyed. This is evident from data reported by Ellenberger[10] on 15 samples of gelatin secured from manufacturers and dealers; when dissolved at 51.7° to 54.4° C. (125° to 130° F.) the bacterial contents per gram of dry gelatin varied from 430 to 2,396,000 and averaged 388,162, when dissolved at 71.1° to 76.7° C. (160° to 170° F.) they varied from 35 to 5,720 and averaged 1,172 while when dissolved in boiling water they ranged from 5 to 3,200 and averaged 530. Brannon and Tracy [11] found that heating gelatin to from 60° to 71.1° C. (140° to 160° F.) greatly reduced the number of bacteria present. Fay and Olson [12] pasteurized 22 samples of gelatin at 62.8° C. (145° F.) for 30 minutes and noted that the efficiency of the heating varied from 0 to 99.9 per cent. With one exception all of the samples containing more than 3,000 bacteria per gram originally showed a reduction of over 90 per cent as a result of the heating. The largest number that survived the heating in any of the samples was 62,000 per gram.

---

[7] Ice Cream Review 7. P. 58. Aug., 1923.

[8] Jr. Dairy Sc. 8. P. 115. 1925.

[9] Jr. Bact. 14. P. 363. 1927.

[10] See ref. 5.

If gelatin is held dry and is properly protected from dust and dirt there is ordinarily little change in the number of organisms present, while if it is stored in a damp place there may be some increase.

Sugar. The bacterial content of sugar can be determind by following the procedure used with gelatin. In general this product contains only small numbers of bacteria and is of little importance as a source of the organisms in ice cream. Ellenberger [13] secured counts that varied from 20 to 255 per gram while those of Olson and Fay [14] ranged from 20 to 240. When sugar is exposed to dust and dirt the bacterial content can undoubtedly be increased.

Flavoring materials. Most flavoring materials are of negligible importance as a source of the bacteria in ice cream because of the small percentages in which they are used and their low bacterial content. Hammer [15] examined five samples of vanilla extract and found the organisms ranging from 200 to 2,300 per cc. Some of the fresh fruits may carry considerable numbers of organisms and the same thing is occasionally true of preserved fruits, especially when they are allowed to stand after opening. The acid prevents the growth of many of the bacteria so that yeasts and molds are commonly the important groups of organisms present.

CONTAMINATION INCIDENT TO THE MANUFACTURE AND HANDLING

The contamination incident to the manufacture and handling of ice cream involves the utensils and equipment, the employees and the air.

---

[11] See ref. 8.

[12] See ref. 9.

[13] See ref. 5.

[14] Jr. Dairy Sc. 8. P. 415. 1925.

[15] See ref. 6.

Contamination from utensils and equipment. The importance of unsterilized utensils and equipment as a source of the organisms in dairy products is now generally recognized. In an ice cream plant the cans, vats, etc. must be considered in this connection but the freezers and homogenizers are especially serious sources of contamination because of the more or less complicated construction.

Hammer and Goss [16] studied the contamination from a freezer by adding sterile water and then operating it for about the freezing time, after which the bacterial content of the water was determined; the freezer was being carefully washed after use but not before the next freezing period. In five trials the bacterial counts per cc. of added water were as follows: 3,700; 141,500; 8,050; 1,195; 300. The increase per cc. of an ice cream mix would have been more than with the water because the latter did not hold air and larger volumes had to be added to fill the freezer as full as an ice cream mix would have filled it. Ellenberger [17] checked the bacteriological condition of a thoroughly steamed freezer by running 500 cc. of sterile water through it and then plating portions of the water; the counts varied from 10 to 55 per cc. and averaged 28. Fay [18] reported an instance in which, under plant conditions, the mix before being placed in the freezer contained 26,000 bacteria per gram while, after freezing, it contained 625,000; he considered that some of the increase may have been due to the breaking up of clumps but that most of it was probably the result of a poorly washed freezer.

The contamination from a homogenizer is very extensive unless proper methods of treatment are employed and, from the standpoint of numbers, the homogenizers constitute the most important source of organisms in the ice cream plant. The organisms are gradually washed out of a homogenizer so that the first material through the machine is the most heavily contaminated.

Employees. The employees in an ice cream plant are one of the sources of the organisms in the finished product. The extent of such contamination depends principally on the personal habits of the individuals involved. Organisms coming from human sources are especially objectionable because of the possibility of pathogenic types being included and, with the modern methods of making ice cream, carriers and cases of disease among the plant employees constitute the most important source of such organisms.

[16] Ia. Agr. Expt. Sta. Bul. 174. 1917.

[17] See ref. 5.

[18] Jr. Dairy Sc. 6. P. 283. 1923.

Air contamination. The contamination of dairy products from the air is now recognized as being less important than was at one time supposed although even under very careful conditions some organisms come from this source. Fabian[19] investigated the contamination from the air in an ice cream plant and concluded that it was insignificant. The majority of the bacteria found in the air were peptonizers, alkali formers or inert types; there were a few weak acid formers but practically no strong acid formers. Fewer molds than bacteria were found. The factors increasing the organisms in the air in what was considered their order of importance were (1) dry and windy weather, (2) open doors and windows, (3) dry floors and (4) machinery running. This investigator found that the amount of air ordinarily entering a freezer during freezing and whipping was small and that a normal overrun could be secured with all the openings to the freezer closed air tight.

CHANGES IN THE NUMBERS OF BACTERIA DURING THE MANUFACTURE OF ICE CREAM

A number of the processes used in the manufacture of ice cream have an influence on the contained bacteria. Some of them cause decreases but others commonly result in increases, as determined by the plate method, because of the breaking up of clumps of organisms.

Making the mix. Results have been secured which indicate that the bacterial content per cubic centimeter of a mix may be less than that of the cream from which it was prepared. This relationship was noted in a number of trials carried out by Hammer and Sanders;[20] a portion of their results are as follows:

Bacteria in Cream and in the Mix Made from It

Type of Cream	Bacteria per CC.	
	Cream	Mix
Pasteurized and held 2 days at about 10° C. (50° F.)	81,500	56,000
Pasteurized and held 1 day at about 10° C.	720,000	156,500
A mixture of old pasteurized lots	5,000,000	4,400,000
Pasteurized and held 3 days at about 10° C.	2,595,000	1,050,000
Raw	24,700,000	15,300,000

---

[19] Mich. Agr. Expt. Sta. Tech. Bul. 83. 1927.

[20] Ia. Agr. Expt. Sta. Bul. 186. 1919.

In eight trials the bacterial content of the mix was from 8 to 75 per cent (av. 43.6%) lower than that of the cream from which it was made.

The smaller number of bacteria in the mix than in the cream may be due, in part, to a volume change, as a result of adding the other ingredients to the cream, but the extent of the decrease in many instances is such that other factors must be operative. One of these is undoubtedly the plasmolyzing action of the sugar which makes up from 12 to 16 per cent of the ordinary mix. This plasmolyzing action undoubtedly varies with different types of organisms so that significant decreases in the numbers present can not always be expected when a mix is made and it is probable that with certain mixes no decrease occurs. Gelatin with an excessive number of organisms is a factor that may tend toward a higher count in the mix but with the usual percentage in which this ingredient is employed no great effect from it can be expected.

Pasteurizing the mix. Pasteurization of the ice cream mix commonly results in a high destruction of organisms. Hammer and Sanders,[21] using pasteurization exposures of from 61.1° to 65.6° C. (142° to 150° F.) for 20 minutes, found that in nine trials the counts after pasteurization ranged from 170 to 66,000 per cc. while before pasteurization they ranged from 56,000 to 15,300,000; a considerable destruction of bacteria had occurred by the time the pasteurization temperature was reached. These investigators suggested that the high concentration of sugar in the mix may have an influence on the pasteurization efficiency secured. Fabian and Cromley[22] in 46 trials found that pasteurization at 65.6° C. for 30 minutes destroyed from 94.5 to 99.9 per cent of the organisms in the mix with an average of 98.97 per cent; after pasteurization the counts ranged from 200 to 50,000 per cc. while before pasteurization they varied from 50,000 to 5,200,000. In the comparisons made by Fay and Olson[23] the mix before pasteurization averaged 17,261,926 per gram while after heating, usually to about 65.6° C. for 30 minutes, the counts averaged 219,953. The average bacterial efficiency was 98.69 per cent and the extremes 86.10 and 99.99.

The pasteurization exposures that are used with ice cream mixes vary rather widely. There is a tendency at present to employ comparatively high exposures in order to secure a pronounced destruction of organisms and there is no disadvantage in this since the effect on the physical condition of the fat is overcome by homogenization and the heated flavor is largely masked by the flavoring materials.

---

[21] See ref. 20.

[22] Mich. Agr. Expt. Sta. Tech. Bul. 60. 1923.

[23] Jr. Dairy Sc. 7. P. 330. 1924.

Homogenizing the mix. Homogenizing the mix usually results in an increase in the bacterial content as determined by the plate method. This may be due, in part, to contamination from the machine but the breaking up of clumps is also a factor.

Fabian and Cromley[24] made counts on the mix following pasteurization and again after homogenization at from 2,500 and 3,000 pounds pressure. In 74 per cent of the 46 comparisons there was an increase ranging from 5.9 to 1,400 per cent, in 10.8 per cent there was a decrease ranging from 9.1 to 86.2 per cent while in 15.2 per cent of the comparisons there was no change. Fay and Olson[25] in 28 trials found an average bacterial content of 219,953 per gram before homogenization and 277,475 after. There was an increase in the count in 18 runs, a decrease in 9 and no change in 1; the average of all the counts showed an increase of 26 per cent as a result of homogenization. Peterson and Tracy[26] in a study of the condensation process of preparing an ice cream mix noted that in the two runs studied homogenization increased the count.

Aging the mix. From the data available on the storage of milk and cream it would be expected that a properly cooled mix could be aged for a reasonable time without a significant increase in organisms. Fay and Olson[27] secured data indicating that, in general, aging a mix 16 to 24 hours does not affect the bacterial count very markedly when the temperature is kept below 7.2° C. (45° F.); the average count of 28 mixes before aging was 191,782 and after aging 192,362 per gram. A mix made by the condensation process was held at from 0° to 1.7° C. (32° to 35° F.) by Peterson and Tracy;[28] the original count was 1,400 per cc., after 5 days it was 1,700, after 14 days 762,000, after 23 days 42,210,000 and after 32 days 188,500,000. These results show the development that may be expected in mixes held for excessive periods even at satisfactory temperatures.

An ice cream mix that is to be aged should be promptly cooled to a low temperature. When a mix that is comparatively warm is put in a cooler, the temperature falls very slowly and the growth of bacteria is thus favored.

Freezing. The freezing of an ice cream mix commonly results in an increase in the bacterial content, as determined by the plate method, even when the condition of the freezer is such that it is not a significant source of organisms. In 51 comparisons of the bacteria in the mix and in the frozen but unhardened ice cream Hammer and Goss[29] found that in 43 (84.3%) the count was increased from 2 to 227 per cent (av. 46.3%), in 6 (11.8%) is was decreased from 2 to 31 per cent (av. 13.0%) while in 2 (4.0%) there was no change. Ellenberger[30] found an average increase of 48 per cent in the number of bacteria as a result of freezing. Fabian and Cromley[31] made 41 comparisons of the counts before and after freezing; in 46.3 per cent of them there was an increase, as a result of the freezing, varying from 0.95 to 260 per cent (av. 46.4%), in 36.6 per cent there was a decrease ranging from 3.85 to 59.7 per cent (av. 25.95%) while in 17.1 per cent there was no change. In 28 trials Fay and Olson[32] found the average count of the mix was 192,362 bacteria per gram while that of the frozen ice cream was 236,688; in only 6 of the comparisons were decreases noted and 4 of these were small enough to be within the limit of experimental error.

---

[24] See ref. 22.

[25] See ref. 23.

[26] Jr. Dairy Sc. 5. P. 273. 1922.

[27] See ref. 23.

[28] See ref. 26.

The increase in the bacterial content which is so frequently obtained during the freezing of an ice cream mix is undoubtedly due to a breaking up of the clumps of bacteria during the agitation in the freezer and should, accordingly, be regarded as an apparent increase. The lowering of the temperature during freezing would be expected to have a destructive influence on certain of the organisms present and this may account for the rather large decreases sometimes noted. The effect of freezing on the bacterial content of a mix is a combination of two distinct and opposing influences, one tending to increase the plate count and the other to decrease it. Each of these influences must have a variable effect, depending especially on the types of organisms present, but the plate counts are most often increased during freezing and the agitation is accordingly the more important.

Hardening ice cream. The hardening of ice cream after it comes from the freezer involves a pronounced decrease in temperature and this would be expected to destroy some of the organisms, the extent of the destruction being dependent on the types present. Hammer and Goss[33] made 52 comparisons of the bacterial content of unhardened and hardened ice cream; in 45 (86.5%) hardening caused decreases ranging from 2 to 75 per cent (av. 39.1%), in 6 (11.5%) it caused increases from 7 to 22 per cent (av. 13.8%) while in 1 (2%) there was no change. The few increases were probably due to experimental error since there was no opportunity for the addition of organisms. Fay and Olson [34] found in 28 comparisons that the bacterial content before hardening was 236,688 per gram while after two or three days storage it was 186,320; increases occurred in 8 of the trials. These investigators consider that the few increases were due to experimental error.

---

[29] See ref. 16.

[30] See ref. 5.

[31] See ref. 22.

[32] See ref. 23.

[33] See ref. 16.

Storage of ice cream. The storage of ice cream is ordinarily carried out at temperatures from somewhat below —17.8° C. (0° F.) to somewhat above. Under these conditions the growth of microorganisms would not be expected. Hammer [35] reported bacterial counts which showed that, in all probability, there is a decrease or else very little change in the numbers of bacteria in ice cream during storage; he also pointed out certain objections to data that had been presented in the literature with the idea of showing an increase. Esten and Mason [36] concluded from their studies of 12 samples that, when ice cream is kept frozen for periods of at least a month, there is no marked increase or decrease in the bacteria, as shown by litmus lactose gelatin plates. Hammer and Goss [37] studied 39 samples of ice cream held by packing in ice and salt and 12 held in a commercial hardening room and found no evidence of an increase in the numbers of contained bacteria, while commonly there was a decrease. The investigations of Ellenberger [38] led to the following conclusion: "There is no radical change in the total number of bacteria in ice cream during storage. There seems, however, to be a tendency toward a slight decrease during the first two to four days, with a more noticeable increase and a corresponding decrease again between the fourth and twenty-first day, after which there is a very gradual falling off in numbers." This investigator also found that the groups of bacteria in ice cream, as determined by litmus gelatin plates and litmus milk tubes, do not change noticeably during storage. Fabian and Cromley [39] concluded that storage of ice cream at an average of —20.5° C. (—4.9° F.) for from one day to three weeks decreases the

[34] See ref. 23.

[35] See ref. 6.

[36] Conn. (Storrs) Agr. Expt. Sta. Bul. 83. 1915.

[37] See ref. 16.

[38] See ref. 5.

[39] See ref. 22.

plate count in the majority of cases although there may be variations in individual samples.

In connection with the changes in the bacterial content of ice cream during storage, the effect of softening and rehardening should be considered. Hammer and Goss[40] reported that this procedure may result in either a significant increase or a decrease in the numbers of bacteria present, the effect presumably being dependent on the types of bacteria and the extent of the softening. An increase seemed to be more common in ice cream which was only slightly softened while a decrease followed pronounced softening, probably because of the more destructive action of the rehardening. Representative results showing the effect of softening and rehardening are as follows:

EFFECT OF SOFTENING AND REHARDENING ON THE BACTERIAL CONTENT OF ICE CREAM

Bacteria per CC. Before Softening	Condition of Softening	Bacteria per CC. After Softening and Rehardening
310,000		368,000,000
2,170,000		6,450,000
156,500		11,450,000
34,000		71,500
360,000	Temperature up to —1.9° C. (28.5° F.). Very icy when rehardened	330,000
470,000	Temperature up to .0° C. (32.0° F.). Extremely icy when rehardened	320,000
26,000	Temperature up to —1.1° C. (30.0° F.). Extremely icy when rehardened	18,000
150,000	Temperature up to —3.1° C. (26.5° F.)	410,000

Some of the extensive increases in the bacterial content of ice cream resulting from the softening and rehardening would be very important if a bacterial standard were being enforced.

Manufacture of ice cream with a low bacterial count. The use of methods that tend to yield ice cream with a low bacterial count is now widespread among well managed ice cream plants. High quality ingredients are carefully handled in properly sterilized equipment with just as little opportunity for contamination from human sources as possible so that a clean and safe product can be put on the market. The method of pasteurizing and homogenizing the entire mix, with the exception of the flavoring material and in some instances the gelatin, has advantages from the sanitary standpoint since it involves the destruction of organisms in ingredients other than the cream and there is less handling after pasteurization and, accordingly, less opportunity for contamination from the utensils, employees, etc.

---

[40] See ref. 16.

In attempts to produce ice cream with a small number of bacteria Hammer[41] secured some counts under 10,000 per cc. The ice cream submitted to various scoring contests quite regularly shows very low counts, even as low as one or two thousand. With methods involving only an ordinary amount of care it seems to be possible to keep the count of the ice cream below 100,000 per cc. most, if not all, of the time. The factor over which the manufacturer has the least control appears to be the cream and, with certain lots containing many organisms that are resistant to pasteurization exposures, the counts may run over 100,000 per cc. Fabian[42] reported that records kept at one large plant over a period of three years demonstrated that the bacterial content of the ice cream could be controlled.

ADDITIONAL CONSIDERATIONS

Bacterial content of sherbets. The bacterial content of water sherbets is ordinarily low compared to that of ice cream. Hammer and Goss[43] examined 17 samples of various flavors and noted counts from 6 to 7,800 per cc. The agar used in plating was commonly clouded by the acid present in the considerable quantity of sherbet added to the plates and the colonies were small. The low counts in water sherbets are in agreement with the statement that the milk derivatives are the most important source of the bacteria in ice cream.

Ice-cream-borne epidemics. Among dairy products ice cream undoubtedly ranks next to milk as a cause of epidemics. For the most part it is consumed without extended holding periods and, as was pointed out in Chapter VII, the low holding temperatures do not free it from pathogenic organisms. Following investigations of two typhoid fever epidemics spread through ice cream, Lumsden[44] concluded that this product deserves the serious attention of health officials because of its importance in the spread of various infections. Fabian,[45] in 1926, listed a considerable number of ice-cream-borne epidemics. In certain outbreaks in which milk was the important means of distributing the organisms, ice cream has been also involved in a minor way.

[41] See ref. 6.

[42] Mich. Agr. Expt. Sta. Spec. Bul. 159. 1927.

[43] See ref. 16.

[44] Am. Jr. Pub. Health. 7. P. 1005. 1917.

[45] Am. Jr. Pub. Health. 16. P. 873. 1926.

It is very probable that the improvements made in the methods of manufacturing ice cream, especially the use of pasteurization, will prove important in limiting the spread of diseases through this product in the future. A number of the reported epidemics have involved ice cream made in homes or in small poorly equipped establishments, where there is much less probability of the use of efficient methods than in modern plants.

CHAPTER XII

BACTERIOLOGY OF BUTTER CULTURES

Cultures of organisms in milk or other materials are frequently inoculated into dairy products for the purpose of bringing about desirable changes or controlling those that are undesirable. The practice apparently began with the use of sour milk to hasten the formation of acid in sweet milk and then gradually developed until, at present, cultures of selected organisms are used to produce flavor and aroma in various dairy products, eyes in Swiss cheese, mold growth in cheeses requiring these organisms for their ripening, etc. and to control objectionable fermentations, especially in certain cheeses.

In the butter industry cultures containing two distinct types of organisms are widely used for the purpose of imparting a desirable flavor and aroma to the product; these are called butter cultures or starters. Such cultures are also used in the making of various cheeses and in the margarine industry.

History of butter cultures. The early attempts to control the flavor and aroma development in cream intended for churning consisted of the addition of clean flavored sour milk or buttermilk from a churning of good butter. In 1889, Conn [1] began the publication of a series of reports dealing with the ripening of cream. He early recognized that the process is a complex one and questioned the ability of any one organism to produce what is known as ripened cream. This investigator [2] specifically pointed out that in cream ripening something besides souring takes place. Conn studied the influence of various organisms on the quality of butter. Of 20 species whose effects were reported in 1893 [3] only 3 produced strikingly bad results while the rest gave good butter or had no marked influence; in no case did a single species produce typical ripening and the few acid producing types did not have as good an influence as the alkali formers. In 1896 Conn[4] pointed out that acid and flavor should be distinguished and that, while the acid is developed from the lactose, the flavor probably comes from some other source; a distinction between flavor and aroma was also suggested and the statement made that it is more unusual for an organism to produce aroma than flavor.

---

[1] Conn. (Storrs) Agr. Expt. Sta. An. Rpts.

[2] Conn. (Storrs) Agr. Expt. Sta. 3rd An. Rpt. P. 136. 1890.

[3] Conn. (Storrs) Agr. Expt. Sta. 6th An. Rpt. P. 43. 1893.

The use of cultures of selected bacteria for the ripening of cream was the result of the investigations of Storch.[5] This investigator studied organisms from such sources as buttermilk, butter and ripened cream and found variations in the flavor and aroma produced by different acid forming types. Among seven such organisms isolated from ripened cream obtained at a plant known for the full flavor of its butter there was one which yielded as full an aroma as a ripened cream possesses.

Weigmann[6] also found a variation in the products formed by acid producing bacteria. When there was a full aroma the butter did not have a clean flavor or good keeping quality and when there was a clean flavor and good keeping quality the butter lacked aroma. This investigator, accordingly, suggested the use of mixtures of organisms or an attempt to find cultures which combined the two qualities.

Commercial cultures for cream ripening appear to have become available in Europe in 1890. Their use increased rapidly, especially in Denmark; in 1897,[7] out of 866 butter plants in that country, 802 used cultures. It should be noted that, although the superiority of Danish butter at this time was commonly attributed to the use of cultures, the idea that the influence of pasteurization was greater than that of the cultures was also prevalent. In the United States cultures were studied, especially by Conn,[8] who pointed out that they were not all alike and that, while most were pure cultures, some were mixtures and that one culture contained no less than 10 or 12 species.

Natural butter cultures. At one time butter cultures that were called natural starters were frequently used. They were secured by allowing milk or cream produced under careful conditions to undergo natural souring and then running this material through a number of transfers in pasteurized milk. A common procedure was to permit a number of samples to sour and then select the one with the best flavor and aroma. Buttermilk from a churning of good butter was also used to develop cultures. In some instances satisfactory natural starters were secured but the chances of obtaining a good culture in this way are small. Sometimes natural cultures are good for a number of transfers and then suddenly become poor; this is due to the increased activity of objectionable organisms that, for a time, were controlled by the desirable types.

---

[4] Conn. (Storrs) Agr. Expt. Sta. 9th An. Rpt. P. 17. 1896.

[5] Abst. in Milch-Ztg. 19. P. 304. 1890.

[6] Abst. in Milch-Ztg. 19. P. 593. 1890.

[7] Hansen, Sophus. Milch-Ztg. 26. P. 590. 1897.

[8] Conn. (Storrs) Agr. Expt. Sta. 11th An. Rpt. P. 85. 1898.

Commercial butter cultures. Various laboratories supply butter cultures that are the result of a more or less careful selection by the use of bacteriological methods and these are called commercial cultures. They are supplied either in liquid or powder form.

The usual liquid employed for commercial cultures is milk, although in the past bouillons have also been used. The milk is commonly sterilized in bottles and then inoculated and sent out as the requests for the cultures are received. Coagulation may occur during the transportation of the culture; if it does not, the culture is allowed to stand after it is received and used for inoculation when coagulation occurs. Another procedure is to transfer, under aseptic conditions, small portions of a developed butter culture to the bottles used for transportation. This method permits the culture to grow at a favorable temperature, instead of at the various temperatures encountered during transportation, but there is also a disadvantage in the greater opportunity for over ripening.

The powdered cultures are prepared by taking good butter cultures, adding an inert material such as starch, lactose, milk powder, etc., to take up a considerable part of the water and then drying at a low temperature. The handling necessary in the preparation of a dry culture often introduces foreign bacteria, but under the usual conditions these are gradually eliminated when the desired types begin their active multiplication. The drying kills many of the organisms but those left are in an inactive condition and remain so because of the lack of moisture; they accordingly survive for long periods, much longer than if they could produce excessive acidities.

The advantage of liquid cultures is that the organisms are active and give a good starter after only a few transfers while their disadvantage is that they have poor keeping qualities because the organisms are in a medium in which growth can go on until the products formed influence their activity. Powdered cultures have good keeping qualities, since the organisms are dormant, but this inactive condition results in growth starting off slowly and a considerable number of transfers are necessary to get the cultures in a condition satisfactory for use; the common presence of contaminating organisms is another disadvantage.

Destruction of organisms during drying. Rogers [9] has suggested that in the preparation of powdered cultures the exposure to a high concentration of salts is largely responsible for the destruction of organisms. The following table, taken from his results, shows the per cent moisture and the number of bacteria per gram at various intervals in the drying of a culture.

THE PER CENT MOISTURE AND THE BACTERIA PER GRAM AT INTERVALS DURING THE DRYING OF A CULTURE

Minutes from Beginning of Drying	Per Cent H_2O	Bacteria per Gram	
		Moist Powder	H_2O Free Basis
0	59.05	785,000,000	1,917,000,000
30	48.05	750,000,000	1,443,000,000
60	34.71	963,000,000	1,475,000,000
90	24.05	942,000,000	1,240,000,000
120	10.56	916,000,000	1,024,000,000
150	4.74	351,000,000	368,000,000
1020	3.25	385,000,000	393,000,000

From these data it is evident that at a water content between 5 and 10 per cent there was a pronounced decrease in the number of bacteria. Rogers had cultures dried by a process in which the water was removed from the milk by a spray carried up by a current of warm, dry air; the drying was complete in a short time and took place at a low temperature. Powders prepared from cultures of an active lactic acid species grown in milk showed large numbers of organisms per gram, although there was a pronounced decrease from the numbers that theoretically would have been present had there been no destruction of organisms. Data on 11 powders showed counts varying from 657,500,000 to 8,590,000,000 per gram.

A method of drying cultures by exposing them in a frozen condition over sulphuric acid in a vacuum approximating 0.01 mm. was also tried out by Rogers. The powders prepared in this way were so active that they curdled milk in 17 hours at 30° C. (86° F.) when added in the ratio of one part of powder to 1,000,000 parts of milk.

---

[9] Jr. Inf. Dis. 14. P. 100. 1914.

The number of bacteria in 11 powders varied from 380,000,000 to 12,670,000,000 per gram.

The investigations carried out by Rogers have established a number of points that are of significance in a consideration of powdered cultures. The addition of dibasic potassium phosphate increased the total number of bacteria in a milk culture but the powder made from such a culture was less active than one made from milk without such an addition. The activity of a powder was not increased by neutralizing the culture with calcium carbonate before drying. The loss of activity in a powder was very slow at 0° C. (32° F.) or lower and became more rapid as the temperature increased until at 30° to 37° C. (86° to 98.6° F.) dried cultures of lactic acid bacteria became inactive in a short time. When the moisture content was comparatively high the loss of activity in powders was more rapid than when it was low. Cultures held in a vacuum retained their activity much better than cultures in an atmosphere of nitrogen or hydrogen, while the most rapid loss of activity occurred in an atmosphere of oxygen or air.

PROPAGATION OF BUTTER CULTURES

The handling of the butter cultures is one of the most important procedures in the modern manufacture of butter. Unless cultures are properly made they may be of no value or actually decrease the quality of the butter. The material and labor necessary for their preparation involve considerable expense that is not justified unless a real benefit is derived from them. In the making of good butter, high quality cultures are exceeded in importance only by the quality of the cream.

Success in the making of cultures requires attention to a great many details. The person who hurries carelessly through the various steps can not expect to make high quality cultures regularly and, unless cultures are uniformly good, their value is questionable. A variation in the quality of the cultures results in a variation in the butter and this is very undesirable from the marketing standpoint. The handling of the cultures should have precedence over all the other plant operations and not be left until there is nothing else that needs attention. If the ripening is to be carefully controlled the conditions must be the same from day to day and, when the procedures have once been standardized for a particular plant, the only variable factor should be the amount of inoculation. It is especially important to inoculate the cultures at a definite time each day because intelligent variations in the amount of inoculating material can not be made without a fairly definite ripening period.

Development of mother cultures. The butter cultures carried in small quantities of milk under very careful conditions are called mother cultures. They demand special attention because they are used in developing the large quantities of culture and, accordingly, must be kept vigorous and uniform. A defect in a bulk culture involves only the operations of one day while a defect in a mother culture may be repeated in succeeding transfers.

Mother cultures are carried in various types of containers, such as milk bottles, glass fruit jars, glass stoppered bottles, special flasks covered with waterproof paper, etc. Containers made of materials other than glass are not advisable as they limit observations and, if metal is exposed to the culture, it may be attacked. Small mouth containers have the advantage of offering a very limited area through which material from the air can enter. Approximately the same amount of milk should always be used in the containers since this aids in controlling the rate of inoculation.

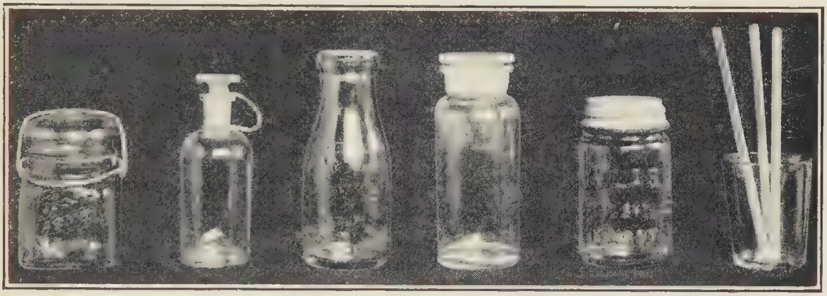


FIG. 26.—Containers for carrying mother cultures.

Milk to be used for butter cultures should be of a high quality, both as to flavor and aroma and as to bacterial content. While certain off flavors and aromas may be largely eliminated by the pasteurization or covered up by the heated flavor developed, many of them carry through into the ripened cultures and are very objectionable. Milk with a high bacterial content can not, on the average, be as completely freed from organisms as milk with a low bacterial content and, occasionally, organisms resisting the pasteurization are responsible for abnormal conditions in the cultures; moreover, excessive numbers of organisms in the milk may be the cause of an abnormal flavor and aroma in it. High quality milk is very essential in the successful handling of cultures.

The milk may be pasteurized and then added to a sterilized container but such handling after heating affords an opportunity for contamination and it is preferable to pasteurize the milk in the container in which it is to be used. A satisfactory procedure is to stand the container, to which the milk has been added, in water reaching up over the level of the milk and then heat the water with steam or a flame. A galvanized iron or copper box with a perforated false bottom and a cover is very useful in carrying out this method; the size should be determined by the number of mother cultures carried and the type of container used for them. The milk should reach 82.2° to 93.3° C. (180° to 200° F.) and should be held for at least 30 minutes. It is then cooled by slowly running cold water into the water around the containers, care being taken to lower the temperature only gradually so as to prevent breakage; slow cooling is especially necessary if the containers have thick walls. The milk should be cooled to from 21.1° to 22.2° C. (70° to 72° F.) and then the commercial culture added and thoroughly distributed. Powdered cultures, with which coagulation is ordinarily rather slow, are sometimes set at temperatures above those usually used for cultures in

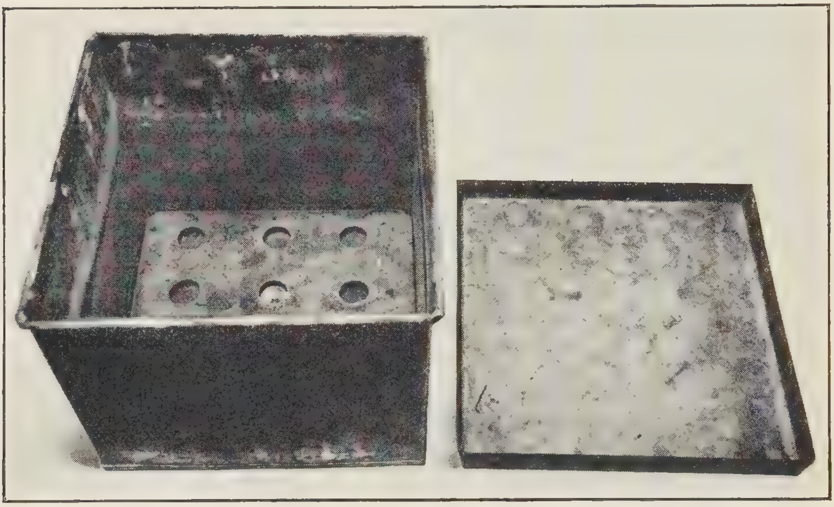


Fig. 27.—Tank with false bottom for heating containers of milk to be made into mother cultures.

order to bring about more rapid coagulation, but acid development is not the only factor involved and there is the possibility that, with this procedure, one of the desirable types of organisms present in the culture may be overgrown. The temperature of the inoculated milk should be held as constant as possible. As soon as the mother culture has definitely coagulated it should be cooled, unless it is to be used for inoculation at once, in order to slow up the action of the contained organisms.

A second mother culture is developed from the first by inoculating a small portion into a lot of milk prepared in the same way as that used for the original culture. The inoculation should be made under careful conditions so that contamination will not occur. Pouring over the lip of the container is not satisfactory because of the danger of adding foreign organisms and the difficulty of getting a definite idea of the amount of inoculating material added. A satisfactory method is to use glass tubes or pipettes that have been sterilized by boiling water, steam, or dry heat; with these there is little chance for contamination, the inoculations can be kept uniform or varied in any way that seems desirable and the cultures can be tasted by drawing a small amount into the mouth. Heated spoons are sometimes used for making inoculations but are less satisfactory than tubes or pipettes and may be responsible for a metallic taste when they are used in judging the cultures.

The series of mother cultures begun in this way is continued as long as the quality is satisfactory. Inoculations are commonly made daily, but a day may be skipped occasionally without objectionable effects, if the ripened cultures are kept cold.

Amount of inoculating material to use. The amount of inoculating material to use is so variable that definite statements with reference to it can not be made. It depends on the ripening period, the activity of the culture, the accuracy of the temperature control and various other factors. The ripening period is largely determined by the time of receiving the culture milk and may be either from morning until late afternoon or from late afternoon or evening until the next morning.

The usual procedure is to inoculate the second lot of pasteurized milk with from 0.25 to 1 per cent of culture taken from the first inoculation. From then on the amount is varied on the basis of the extent of the ripening secured. Changes are frequently made as the culture appears to be under or over ripe and, for this reason, approximate measurements of the inoculating material used are necessary. The rate of ripening should be controlled entirely by the amount of inoculation and never by varying the temperature.

Controlling the temperature of developing mother cultures. Various methods of maintaining, or attempting to maintain, satisfactory incubation temperatures are used with mother cultures. At the one extreme is the system of placing the inoculated milk in the location where the temperature seems to be the least objectionable, for example, the boiler room in winter and an open window on a warm summer night, while at the other extreme are the automatically operated controllers which are heated with electricity and cooled with ice or brine. There are also various simple systems of heating an insulated cabinet with an electric light or other resistance that are

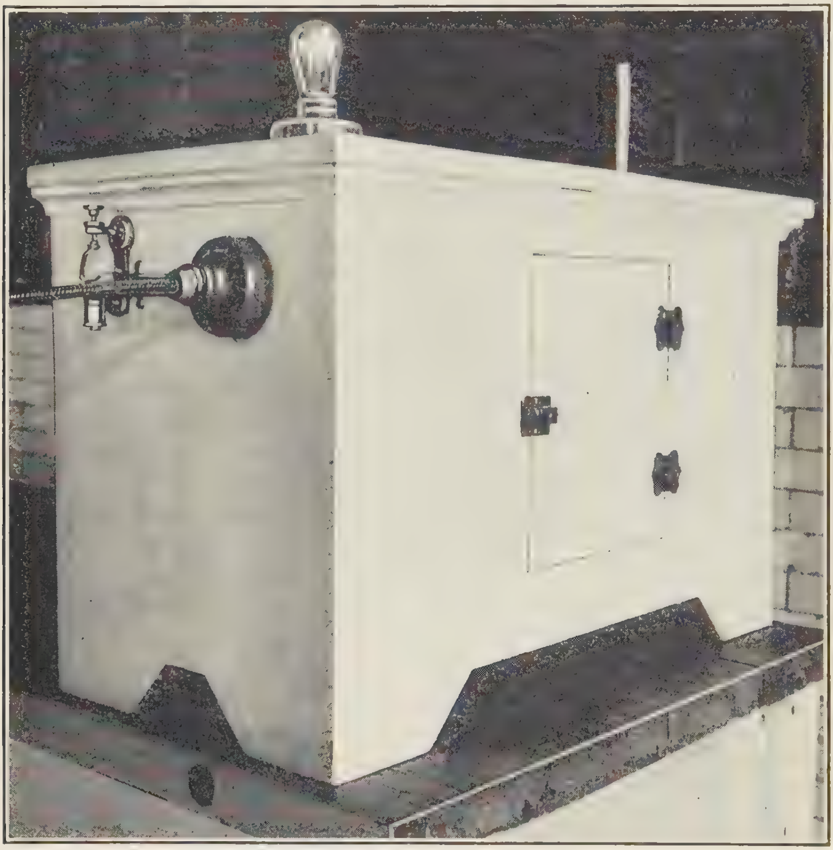


Fig. 28.—Home-made temperature controller for mother cultures.

very satisfactory; in summer, when the room temperature is higher than that desired, the cabinet can be held in the cooler. In many instances a well insulated container, such as an ice cream cabinet or a specially made box, has proved quite satisfactory for holding the mother cultures, especially when they are surrounded with a large volume of water to delay temperature changes.

Every plant using butter cultures should have some sort of a temperature controller, the exact type to be determined by the expenditure that is decided on. Home made equipment is often very satisfactory, the exact efficiency being dependent principally on the ingenuity of the builder. The question of the sanitation should be considered so that the equipment can be kept free from the odors coming from wet wood or insulating material. A metal lining for the holding compartment is advisable and absolutely necessary if a volume of water is used to delay temperature changes.

Over ripening. The amount of acid present when a culture coagulates varies somewhat, due presumably to differences in the composition of the milk, etc. It may be as low as 0.5 per cent but is usually from 0.65 to 0.70 per cent. Definite coagulation is a good basis on which to stop the development of cultures by cooling, since it can be determined without opening the container and thus without danger of contamination. If cultures are allowed to stand for considerable periods at a temperature favorable for growth the acidity may go up to 1.1 per cent or even higher. Excessive acid development results in more or less characteristic undesirable flavors and aromas that interfere with the judging; it may also have an influence on the organisms present so that their action is less desirable.

Moderate over ripening apparently does not influence the ability of a culture to coagulate rapidly milk into which it is inoculated; Hammer and Baker[10] found that more than 30 hours holding at 21.1° C. (70° F.) after coagulation did not appreciably decrease the rate at which cultures coagulated milk, as compared to the rate at the time of curdling. There is, however, the possibility of over ripening interfering with the relationship between the two types of organisms.

A certain amount of acid must be present in a culture before the desirable flavor is developed and, at low acidities, cultures regularly lack flavor. In accordance with this, Cordes and Hammer[11] found that at low acidities the per cent of the total acid made up of volatile

[10] Ia. Agr. Expt. Sta. Res. Bul. 106. Part 2. 1928.

[11] Ia. Agr. Expt. Sta. Res. Bul. 66. 1921.

acid was also low and that, as the total acid increased, this percentage increased. Occasionally cultures continue to lack flavor even when considerable amounts of acid have been developed.

Preparation of large volumes of butter culture. Large volumes of butter culture are prepared in culture cans or vats, using the same general procedure as is used with mother cultures. The inoculations are made directly from mother cultures or, if large quantities are required, special cultures for inoculation may be prepared in enamelled cans. The amount of inoculating material to be used is more difficult to determine with a large volume of milk than with a mother culture because of the variations in the quantity from day to day and the greater fluctuations in the ripening temperature.

JUDGING BUTTER CULTURES

The ability to accurately judge the quality of butter cultures is very necessary for the person handling them. Both the desirable and undesirable characters must be recognized and the causes of the objectionable conditions should be understood.

General appearance. Cultures may be examined for their general appearance at any time but the remainder of the examination should not be carried out until transfers have been made because of the possibility of contamination. If gas formation has occurred bubbles are usually present near the bottom of the culture but if the gas is abundant they may be distributed throughout. Furrows made by bubbles passing to the surface are sometimes evident next to the wall of the container. In some instances gas forming organisms can be eliminated from a culture by a series of transfers but the process is rather uncertain and a gassy culture should be discarded. Whey at the surface of a culture is of no great significance. It may be due to a high acidity, although often there is a high acidity without the presence of whey. If a culture is agitated, even slightly, at about the time of coagulation, wheying off frequently occurs.

Texture, body, flavor and aroma. When a culture is to be examined for texture, body, flavor and aroma it is first thoroughly broken up by shaking or stirring. The texture and body are quickly judged when a small amount is poured out. The texture should be smooth and creamy without any suggestion of lumps; there are undoubtedly various causes for an uneven texture but one that has been definitely established is an incubation temperature that is much too high. The body should be of such a character that when the culture is poured on a flat surface it stands up well and has a velvety appearance. A thin body may be due to a low acidity but is frequently caused by contaminating organisms. A culture may show ropiness and, when this occurs, *S. lactis var. hollandicus* can ordinarily be isolated. With some cultures ropiness appears and disappears for no apparent reason. A ropy culture may have a satisfactory flavor and aroma but the ropiness is an indication of instability and, accordingly, the culture should be discarded. Occasionally, when a culture is poured from the container it is found to have a dull, chalk-white appearance; the common cause of this is over ripening.

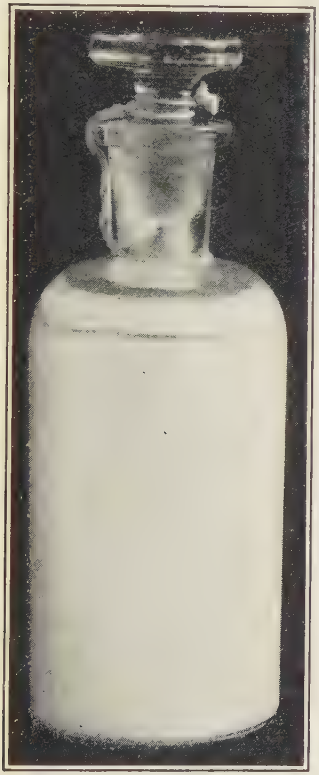


FIG. 29.—Mother culture showing whey beneath the cream layer.

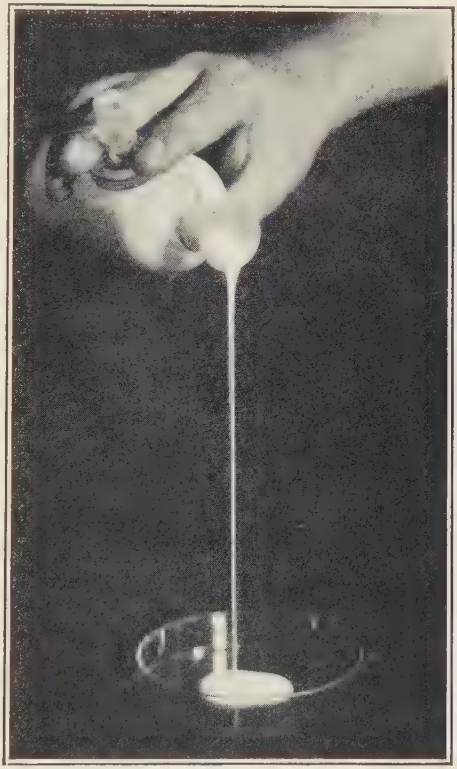


FIG. 30.—Ropy mother culture.

The flavor and aroma of a butter culture should be delicate and pleasing and free from any suggestion of an objectionable condition. If a culture is insufficiently ripened it will be lacking in flavor and aroma, while if over ripened the flavor and aroma will not be delicate but coarse. There does not appear to be a definite acidity which is always the most desirable and some cultures must be ripened more than others to bring out the desired condition; with a given culture there may also be a variation from day to day. A full aroma is more difficult to secure than a satisfactory flavor. In some instances a culture lacks flavor and aroma because one of the types of organisms develops poorly, or not at all, and under these conditions no amount of ripening will bring out the desired flavor and aroma.

Abnormal flavors and aromas. A number of objectionable flavors and aromas, such as barny, unclean, bitter, yeasty, rancid, etc., are encountered in butter cultures. These are the result either of some condition of the original milk or of the activity of undesirable organisms that gain entrance. The proper selection of milk on the basis of freedom from off flavors and aromas is very essential if a good product is to be secured. The contaminating organisms that cause objectionable flavors and aromas in butter cultures come from various sources, such as unsterile transfer equipment or contamination from the mouth of the container, and when once present in a culture may be carried along from one transfer to the next. Differences in the extent of their growth from day to day may result in variations in the intensity of the condition caused by them; the condition may even seem to entirely disappear and then later be very evident. With some cultures contaminating organisms can readily be detected by microscopic examination, but plate or slope cultures, using whey or beef infusion agar, are to be preferred.

Conditions to observe in judging cultures. In judging the flavors and aromas of cultures it is essential that the temperature be approximately uniform from day to day if accurate comparisons are to be made. The person who is accustomed to examining cultures at fairly low temperatures has difficulty in judging those that have not been cooled. When a culture is poured out into a glass or cup it should be judged at once as free contact with air for only a few minutes may have a striking influence on the flavor and aroma. There is a distinct advantage in having a number of cultures to compare and they should differ in quality. When only one culture is examined day after day it is difficult to recognize small but significant defects and often the culture is considered to be much better than comparisons would show it to be. Another advantage in carrying a number of cultures is that there is more assurance of having satisfactory inoculating material since it is improbable that all of the cultures will develop off conditions at the same time unless there has been some general defect in the method of propagation, such as an unsuitable incubation temperature or improper pasteurization of the milk.

INFLUENCE OF VARIOUS FACTORS ON BUTTER CULTURES

A great many factors have an influence on the quality and general condition of butter cultures. Some of these have been studied and their effect recognized so that they can be taken into account in the handling of cultures but there are undoubtedly others still unknown that may be of sufficient importance to explain the variations that occur from day to day in the quality of cultures.

Influence of the temperature of incubation. Under practical conditions, where the ripening temperature is often quite variable, satisfactory butter cultures are secured over a considerable temperature range. Toens and Hammer[12] used a number of temperatures and found that good cultures could be secured at as low as 18° C. (64.4° F.) and at as high as 32° C. (89.6° F.); at 37° C. (98.6° F.) the flavor and aroma were very unsatisfactory and the fermented milk did not resemble a butter culture, although sufficient acid was regularly developed. The temperature usually used for ripening—21.1° to 22.2° C. (70° to 72° F.)—has advantages of considerable importance. When the temperature is lower than this the growth of the organisms is delayed, while when it is higher conditions are more favorable for the development of the heat resistant bacteria that survive the pasteurization. The failure to secure good results at comparatively high temperatures indicates that at least one of the normal culture organisms finds conditions unsatisfactory for growth.

Constant and irregular ripening temperatures. Under practical conditions there is often considerable fluctuation in the ripening temperature. Extreme variations are clearly undesirable, because of their pronounced influence on the rate of ripening, but the effect of smaller variations is not so evident. Toens and Hammer[13] studied the influence of a changing temperature on the quality of a starter in a series of comparisons in which one culture was maintained at a constant temperature while the temperature of the other, which had been similarly prepared, was allowed to fall. The setting temperature used was 32° C. (89.6° F.); this was known to give satisfactory cultures under the conditions employed and had the advantage of permitting considerable drops in temperature. In the 87 comparisons the constant temperature culture was the better in 61 (70%), while the fluctuating temperature culture was preferred in 26 (30%). These results suggest that constant temperatures are desirable in the propagation of butter cultures.

---

[12] Ia. Agr. Expt. Sta. Res. Bul. 85. Part 1. 1925.

[13] See ref. 12.

Influence of the length of the ripening period. The ripening periods usually used with butter cultures vary from 6 or 8 hours to 18 or 20 hours and satisfactory results are secured in plants using very different periods. Toens and Hammer[14] compared rapid (6 to 8 hours) and slow (16 to 20 hours) coagulations in 25 trials with each of three starters, the time of coagulation being controlled entirely by varying the amount of inoculation. Although the differences were often small, the rapidly coagulated culture was considered the better in 64 (85%) of the 75 comparisons while the slowly coagulated culture was placed first in 11 (15%); the comparisons with each starter in which the rapidly coagulated culture was placed first were 92, 76 and 88 per cent respectively. The advantage of rapid coagulation may be due to the lessened chance for the growth of the organisms resisting high pasteurization since, for the most part, these types are rather susceptible to acid and their development would be quickly interfered with. Another factor appears to be that with a short ripening period there is less danger of pronounced over ripening than with a long period.

Influence of the milk used. Whole milk with a good flavor and aroma is the most satisfactory medium for the development of butter cultures. Skim milk has been extensively used but ordinarily does not give as full and delicate a flavor as whole milk and there is little economy in using it since the fat in the whole milk is recovered as butter during the churning of the cream in which the butter culture is employed. With milk made up from milk powder or evaporated milk and inoculated after pasteurization, coagulation is often very slow and the curd may be less firm than that normally secured; the conspicuous heated flavor also makes the judging very difficult.

Baker and Hammer[15] investigated the modification of milk and found that the addition of lactose, fat or water yielded cultures with a lower acidity and a less satisfactory flavor and aroma than normal milk. Milk ash gave a salty taste that was not objectionable and tended to bring out the desirable flavor. None of the milk modifica-

[14] See ref. 12.

[15] Ia. Agr. Expt. Sta. Res. Bul. 92. 1926.

tions that were tried appeared to be of value in the development of butter culture.

In the studies of Baker and Hammer[16] different lots of normal milk did not give exactly the same results when used in the preparation of butter cultures. With the same rate of inoculation and time of incubation considerable variation occurred in the acidities produced by a culture in lots of milk from individual animals and there was a tendency for the milk from a given animal to maintain the same general ranking as to the acidity produced; in general, milk having a high content of total solids gave a high acidity when it was made into culture but the correlation was not a close one. Considerable variation occurred in the scores of the cultures prepared from lots of milk from individual animals, using inoculating material from the same source, and the milk from a given animal fluctuated from time to time in the quality of the cultures prepared from it; these differences may have been due, in part, to the variations in the acidities of the different cultures. Milk from various herds also showed differences in the acidities developed under definite inoculation and incubation conditions but these were less than the differences with the milk from individual animals.

Since citric acid is a source of the volatile acid produced in a butter culture, the addition of this material to milk to be made into culture seems indicated. Such an addition, however, gives a sharp acid flavor and does not result in an improvement. Small amounts of sodium chloride seem to intensify the normal culture flavor but are apparently of no value in giving the desired flavor to butter.

Influence of the air supply. Although there is not much difference in the time of coagulation between the top and bottom of a volume of milk being made into culture, the bottom regularly coagulates first. The settling out of the inoculating material may be a factor here but the air supply must also be considered. Toens and Hammer[17] compared the rate of acid development in thin layers of milk held in cotton stoppered flasks with the rate in deeper layers held in glass stoppered bottles and found that it was more rapid with a restricted than with an abundant air supply; the effect of the air supply on the flavor and aroma of the culture was not definite and seemed to be dependent, in part, on the conditions under which the mother culture was carried.

Influence of the pasteurization exposure used with the culture milk. The pasteurization exposure used with the milk to be made into culture has an influence on the rate of acid development and coagulation. Hammer and Baker[18] found that when cultures were made from lots of milk pasteurized with different exposures, the milk heated to 62.8° C. (145° F.) for 30 minutes developed acid and coagulated more slowly than that heated to considerably higher temperatures. Essentially the same rate of growth of the culture organisms occurred when the milk was heated to 71.1° C. (160° F.) for 30 minutes as when higher temperatures were used for this period. The variations noted in the rate of coagulation following different pasteurization exposures were undoubtedly due to the effect of heat on the germicidal property of milk. With a heating period of 30 minutes the influence of the heat appeared to be gradual and spread over a considerable temperature range, instead of occurring completely at a definite temperature. These results indicate that the use of high pasteurization exposures with milk intended for cultures has an advantage in addition to the destruction of most of the contained organisms. In this connection a report coming to the Iowa Agricultural Experiment Station is of interest. Because of the lack of time on a certain day the culture milk in one of the Iowa creameries was pasteurized as market milk is pasteurized and then cooled and inoculated in the regular way, with the result that coagulation was much slower than usual.

---

[16] See ref. 15.

[17] See ref. 12.

Influence of holding cooled cultures to be used for inoculation. Mother cultures are commonly cooled as soon as ripe with the idea of keeping the organisms in a condition satisfactory for the development of new cultures. Hammer and Baker[19] studied cultures held in ice water for considerable periods after coagulation and found that the rate at which they induced curdling in milk into which they were inoculated showed no appreciable change; the acidity remained low, which indicates that the growth of the organisms was effectively checked. Freezing ripened mother cultures had no definite influence on the rate of coagulation induced in inoculated milk, although there was some evidence of a decrease in the numbers of organisms.

ORGANISMS NORMALLY PRESENT IN BUTTER CULTURES

At one time the prevailing idea seems to have been that butter cultures contain only lactic acid organisms. There was little evidence for this and some to the contrary since both Conn and Weigmann, as already noted, found it impossible to isolate an organism that, in

[18] Ia. Agr. Expt. Sta. Res. Bul. 106. Part 3. 1928.

[19] See ref. 10.

pure culture, would bring about the change occurring in ripening cream. In 1919, Hammer and Bailey[20] pointed out that butter cultures produce considerable volatile acid in milk, while *S. lactis* does not, and reported the isolation of an organism from butter cultures that yielded considerable volatile acid and which was evidently important from the standpoint of flavor and aroma development. At about the same time Storch[21] noted the presence in butter cultures of organisms which he called X bacteria and Boekhout and Ott de Vries[22] isolated their "aroma" bacteria. Later Ayers and Mudge[23] also found an organism other than *S. lactis* in a butter culture studied by them.

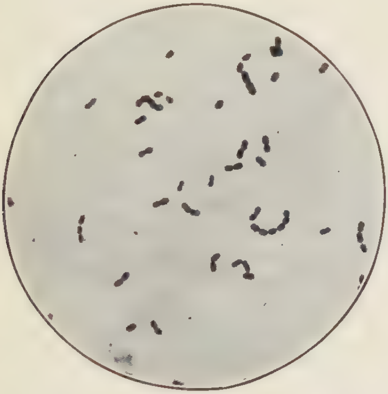


FIG. 31.—Bacteria in a mother culture.

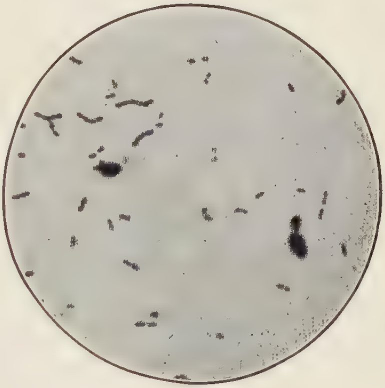


FIG. 32.—Mother culture showing contaminating yeast cells.

The evidence that butter cultures are not pure cultures of *S. lactis* is at present convincing and has been summarized by Hammer[24] as follows:

1. Cultures of *S. lactis* produce only small amounts of volatile acid in milk while butter cultures produce comparatively large amounts.
2. By preparing a series of dilutions of a starter, cultures can be secured which coagulate milk rapidly but which yield only a low volatile acidity like that of *S. lactis*. This indicates that

---

[20] Ia. Agr. Expt. Sta. Res. Bul. 55. 1919.

[21] 102 de Beretning fra. Forsogslaboratoriet. 1919.

[22] Centbl. f. Bakt. 2 Abt. 49. P. 373. 1919.

[23] Jr. Dairy Sc. 4. P. 420. 1921.

[24] Proc. World's Dairy Cong. 2. P. 985. 1923.

an organism responsible for the high volatile acidity in the butter culture has been diluted out.

3. Organisms other than *S. lactis* can be isolated from a good butter culture.
4. Satisfactory butter cultures can not be prepared from pure cultures of *S. lactis* but can be developed from mixtures of *S. lactis* and another organism isolated from butter cultures.
5. Only *d* lactic acid is produced by cultures of *S. lactis* while butter cultures yield some *i* lactic acid.
6. The ratio between the total and volatile acidities in a butter culture is quite different than that in a pure culture of *S. lactis*. Moreover, in the case of a butter culture this ratio changes as the acidity goes up but remains quite constant with a culture of *S. lactis*.
7. *S. lactis* does not cause a decrease in the citric acid content of milk in which it is grown while a butter culture does.
8. The volatile acids distilled from well ripened butter cultures have barium and Duclaux values different than those distilled from pure cultures of *S. lactis*.

The organisms other than *S. lactis* that are present in butter cultures may be conveniently referred to as the "associated" organisms. Hammer[25] has divided these into two types, *Streptococcus citrovorus* and *Streptococcus paracitrovorus*. The fundamental difference between them is that *S. paracitrovorus* produces some lactic acid in milk, while *S. citrovorus* does not. Both organisms produce volatile acid from lactic acid and, accordingly, in pure culture *S. paracitrovorus* develops more volatile acid than *S. citrovorus* because of its lactic acid production. *S. paracitrovorus* reddens litmus milk and occasionally even coagulates it while *S. citrovorus* produces little change in litmus milk other than a slow reduction.

Characters of the associated organisms. The outstanding character of the associated organisms is their fermentation of citric acid with the formation of volatile acid. This can be shown by adding sterile citric acid in solution to sterile milk, inoculating the organism to be studied, incubating and then determining * the volatile acidity and comparing it with that produced under the same conditions in milk alone. Such a procedure shows that the addition of citric acid increases very materially the volatile acid production of the associated organisms. The following table gives representative data illustrating this.

---

[25] Ia. Agr. Expt. Sta. Res. Bul. 63. 1920.

* A satisfactory method of determining the volatile acidity produced consists of distilling a 250 gram portion of milk with steam after the addition of 15 cc. of approximately n/1 H_2SO_4 until 1,000 cc. of distillate have been obtained and then titrating with n/10 alkali, using phenolphthalein as an indicator. The results are expressed as the cc. of n/10 alkali required. This method does not,

The Effect of Citric Acid on the Volatile Acid Production of the Associated Organisms

Trial	Volatile Acidity
1. *S. citrovorus* in milk	21.2
S. citrovorus in milk plus 0.12% citric acid crystals	40.0
S. citrovorus in milk plus 0.25% citric acid crystals	56.1
2. *S. citrovorus* in milk	24.0
S. citrovorus in milk plus 0.12% citric acid crystals	45.6
S. citrovorus in milk plus 0.25% citric acid crystals	62.0
3. *S. paracitrovorus* in milk	30.9
S. paracitrovorus in milk plus 0.40% citric acid crystals	82.5
4. *S. paracitrovorus* in milk	26.8
S. paracitrovorus in milk plus 0.40% citric acid crystals	86.9

The fermentation of citric acid by the associated organisms can also be shown by determinations of the citric acid content of milk before and after the growth of these types; in general, the citric acid is completely destroyed. Before the associated organisms were studied the disappearance of citric acid during the souring of milk was noted by Bosworth and Prucha.[26] Since these organisms are generally present in sour milk or cream they are undoubtedly involved in the destruction of the citric acid during natural souring.

The action of the associated organisms on lactic acid is also of significance from the standpoint of the ripening of cream. The addition of a small amount of sterile lactic acid to milk in which *S. citrovorus* is to be grown results in an increase in the volatile acidity produced and the conclusion naturally follows that lactic acid is, in

---

by any means, recover all the volatile acid but gives results that are of value from a comparative standpoint. Flasks or pint milk bottles containing 325 cc. of milk are very satisfactory for the preparation of cultures for the volatile acid determinations. When the effect of added citric acid is to be studied, from 0.3 to 0.4 per cent of the crystallized acid (1 mol. H_2O) in a sterile aqueous solution should be added to the sterile milk with rapid agitation to prevent, as much as possible, the precipitation of the casein.

[26] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 14. 1910.

part, changed over to volatile acid. It seems very evident, however, that the lactic acid is not such an easily usable source of volatile acid as is citric acid since the addition of lactic acid to milk never yields the high volatile acidities that result from the addition of citric acid. With *S. paracitrovorus* the addition of sterile lactic acid to milk does not increase the volatile acidity produced and this is apparently due to the fact that the organism produces lactic acid which it may later change over, in part, to volatile acid.

The effect of lactic acid on the volatile acid production of *S. citrovorus* in milk is evident from the following data which show that the lactic acid can either be added directly or produced in the milk by *S. lactis.*

THE EFFECT OF LACTIC ACID ON THE VOLATILE ACID PRODUCTION OF *S. citrovorus*

Trial	Volatile Acidity
1. *S. citrovorus* in milk	22.0
S. citrovorus in milk plus 1 cc. sterile lactic acid (about 70%)	31.2
2. *S. citrovorus* in milk	22.0
S. citrovorus in milk plus 1 cc. sterile lactic acid (about 70%)	31.5
3. *S. citrovorus* in milk	15.7
S. lactis in milk	3.7
S. citrovorus and *S. lactis* in milk	34.7
4. *S. citrovorus* in milk	21.3
S. lactis in milk	4.5
S. citrovorus and *S. lactis* in milk	35.8

The chemical changes produced by the associated organisms are the most valuable characters from the standpoint of identification. The morphology gives no information of value, since they stain and appear like other streptococci present in milk, particularly *S. lactis.* The morphologic similarity to *S. lactis* is perhaps an important reason why butter cultures were at one time considered to be pure cultures. In milk and various other media the cultural characters of the associated organisms include nothing that is unusual. For the experienced person the odor produced in milk by *S. paracitrovorus* is more or less characteristic when taken in conjunction with other changes; *S. citrovorus,* however, produces no definite odor due, presumably, to the fixing by the milk constituents of the volatile acids formed.

Both *S. citrovorus* and *S. paracitrovorus* produce gas in milk but the gas production with these organisms is very much less conspicuous than with such typical gas formers as the Escherichia-Aerobacter group and cultures may be studied in a routine way without the gas production being noted. The sealing of freshly inoculated milk cultures, for example, with a mixture of paraffin and vaseline, tends to retain the gas formed and the seal is forced up. The gas production of these organisms can also be shown by inoculating cans of evaporated milk and soldering up the opening through which the inoculation was made; the gas developed ordinarily results in a bulging of the cans. An occasional *S. paracitrovorus* culture is secured which rapidly coagulates milk and this coagulation may be accompanied by the presence of definite bubbles of gas.

Orla-Jensen, Orla-Jensen and Spur[27] have pointed out the favorable influence of yeast extract, even in small amounts, on the growth of the associated organisms and have noted that the acid production from various fermentable materials is stimulated by this.

Isolation of the associated organisms. The isolation of the associated organisms can ordinarily be accomplished by plating. The butter culture, or other material from which the isolation is to be made, may be plated on whey or beef infusion agar, the plates incubated at room temperature and the well developed colonies picked into litmus milk. Cultures which do not show the typical *S. lactis* reaction in litmus milk can be examined for morphology, changes produced in milk and volatile acid production in milk, with and without added citric acid. This method involves considerable work since the great majority of the colonies picked are *S. lactis* and often a large number include no associated organisms. The use of various other plating media has not made possible a differentiation of the two types of organisms on the plates. The comparatively small number of associated organisms present in butter cultures and their uneven distribution make their isolation by direct plating rather difficult; occasionally, however, a considerable percentage of the cultures secured from a plate will be associated organisms.

Other methods may be used for the isolation of the associated organisms. By inoculating butter culture on a whey agar slope and running the developing growth through a number of transfers on the same medium, with several days between transfers, cultures are sometimes secured which do not show the *S. lactis* reaction when inoculated into litmus milk. Occasionally butter cultures, which have been held for considerable periods so that many of the contained organisms are dead, reach a condition in which they yield growth on whey agar slopes but fail to give a *S. lactis* type of development in litmus milk. Cultures secured by either of these methods can be plated to establish the purity and the final cultures selected checked for the characters of the associated organisms.

---

[27] Jr. Bact. 12. P. 333. 1926.

Distribution of the associated organisms. The associated organisms are apparently regularly present in butter cultures of a high quality. Hammer[28] found them in cultures that were being carried in creameries and also in the cultures sent out by laboratories, although those distributed from one source seemed to contain only *S. lactis*. *S. paracitrovorus* has been repeatedly secured from sour milk and cream but *S. citrovorus* is much less commonly found in these materials. Both organisms are occasionally obtained from butter in which case they may represent types derived from the culture used. Certain of the organisms isolated from various cheeses undoubtedly belong to the group represented by the associated organisms.

In some instances in which pure cultures of *S. lactis* have been taken to creameries and carried through a series of transfers the cultures eventually secured have shown a typical butter culture flavor and aroma. This has undoubtedly been due to contamination with the associated organisms in the plants and indicates that these types are widely distributed; it also shows why pure cultures of *S. lactis* can be put out as butter cultures and satisfactory results secured in a certain percentage of cases.

Variations in the associated organisms. Rather striking variations occur in the total and volatile acidities produced by *S. paracitrovorus*, as is shown by the following summary of the results secured by Hammer and Baker[29] on 124 cultures.

Total and Volatile Acidities Secured on 124 Cultures of *S. paracitrovorus*

	Total Acidity Per Cent	Volatile Acidity	
		In Milk	In Milk Plus 0.4 Per Cent Citric Acid Crystals
Minimum..........	0.27	12.5	44.4
Maximum..........	0.99	40.0	96.6
Average...........	0.46	28.6	73.9

---

[28] See ref. 25.

[29] Ia. Agr. Expt. Sta. Res. Bul. 81. 1923.

Some of the *S. paracitrovorus* cultures produced enough acid to coagulate milk while others did not; with an occasional culture coagulation was fairly rapid. A few of the cultures produced ropiness in milk.

Variations in the volatile acidity produced by *S. citrovorus* cultures also occur. Differences in the cultural characters of a number of isolations of this organism were noted at the Iowa Agricultural Experiment Station and were found to be constant over considerable periods; for example, one culture was studied which regularly gave a comparatively heavy growth on whey agar, much heavier than any of the other *S. citrovorus* cultures that were being investigated at the same time.

The variation of greatest concern from the practical standpoint with both of the associated organisms is that occurring in the quality of the starters that are secured with different cultures. Certain cultures have been observed to give a good starter quite regularly while others never seem to. It is worthy of note that the quality of a starter secured with a certain culture of one of the associated organisms is not necessarily good because the volatile acid production is high; this is evidence that the volatile acidity is not the only point of consideration with reference to the aroma and flavor development in butter cultures.

Isomeric form of lactic acid in butter cultures and in cultures of S. paracitrovorus. Hammer[30] found that butter cultures carried under practical conditions contained some inactive lactic acid and that with mixtures of *S. lactis* and *S. paracitrovorus* the percentage of the total lactic acid that was inactive was quite high. The lactic acid produced by *S. paracitrovorus* is of the *l* type.

Volatile acids produced in milk by butter cultures and by the associated organisms. Hammer and Sherwood,[31] using both the Duclaux method and the method of determining the per cent barium in the barium salts, found that the volatile acids contained in butter cultures ripened for considerable periods were made up largely of acetic acid with small amounts of propionic. When the cultures were ripened to low acidities, propionic acid made up a greater percentage of the volatile acid than when they were ripened to high acidities. Studies of the various organisms normally present in cultures showed that the low volatile acid production of *S. lactis* in milk involved acetic acid with considerable amounts of propionic while the volatile acid production in milk by *S. citrovorus* or *S. paracitrovorus* involved very largely acetic acid.

---

[30] Ia. Agr. Expt. Sta. Res. Bul. 65. 1920.

[31] Ia. Agr. Expt. Sta. Res. Bul. 80. 1923.

Importance of lactic acid in butter cultures. The lactic acid produced in butter cultures is important from several standpoints as follows:

1. The lactic acid may be a source of volatile acid. The evidence that volatile acid can be produced from lactic acid suggests that such a transformation occurs during the normal development of butter cultures.
2. The lactic acid tends to free the volatile acids from combinations with the milk constituents. When the volatile acids are combined their effect on the flavor and aroma is much less than when they are free.
3. The lactic acid prevents the growth of undesirable bacteria. The growth of various organisms that occasionally gain entrance to butter cultures is usually controlled by the rapid acid development. The normal culture organisms tolerate considerable acid and, accordingly, can grow under conditions which limit the activity of many species. The influence of the acid is an important factor in controlling objectionable organisms in the butter cultures carried under practical conditions.

The lactic acid present in a butter culture is primarily produced by *S. lactis*. In cultures containing *S. paracitrovorus* some of the lactic acid may be formed by it but the amount can not be very large because of the comparatively slow acid production by this organism. The lactic acid formed by *S. lactis* accordingly appears to play an important part in the development of a butter culture; this suggests that the use of pure cultures of one of the associated organisms in butter plants, instead of the usual butter cultures, is not likely to prove satisfactory.[32]

Proportion of S. lactis and associated organisms in butter cultures. Attempts at the isolation of the associated organisms by direct plating indicate that *S. lactis* is much more numerous in butter cultures than the associated organisms. This can also be shown by inoculating dilutions of a butter culture into flasks of sterile milk and determining the volatile acid production secured with the different amounts; commonly, rapid coagulation occurs with quantities much smaller than those yielding a volatile acidity typical of butter cultures. Orla-Jensen, Orla-Jensen and Spur[33] did not succeed in isolating the associated organisms from most cultures without previous enrichment; their regular presence was shown, however, by the isomeric form of lactic acid produced in milk to which chalk had been added.

---

[32] Ia. Agr. Expt. Sta. Res. Bul. 67. 1921.

Relationship of the associated organisms to other types. The observations on the citric acid fermenting power of the associated organisms have recently been confirmed by Orla-Jensen, Orla-Jensen and Spur[34] and this character seems to be the outstanding one possessed by these streptococci. Because the associated organisms produce gas and volatile acid in milk certain investigators have considered them to be *Streptococcus kefir,* although records on the citric acid fermenting power of authentic cultures of this species appear to be lacking. In this connection it is of interest to note the isolation by Hammer[35] of a Streptococcus which produced considerable volatile acid in milk without fermenting the citric acid.

DEVELOPMENT OF BUTTER CULTURES

With the information available relative to the organisms normally present in butter cultures it is possible to develop new cultures under such conditions that only desirable bacteria are present. The two types of organisms, *S. lactis* and one of the associated species, may be mixed directly in sterile milk or the associated organism may be allowed to grow for a few hours or overnight and *S. lactis* then added. When the mixed culture is coagulated it should be run through a number of transfers in sterile milk and then, when coagulation becomes rapid, it may be used in thoroughly pasteurized milk. All the transfers should be grown at the temperature usually used with butter cultures. The early transfers may coagulate slowly because of the restraining action of the associated organisms on *S. lactis* and, for this reason, the interval between the inoculation of the two types should not be too long; the necessity of having a great many of the associated organisms in order to prevent their overgrowth by *S. lactis* before they become established makes it advisable, however, to allow them to grow alone for a short time.

---

[33] See ref. 27.

[34] See ref. 27.

[35] Ia. State Col. Jr. Sc. 2. P. 1. 1927.

The *S. lactis* culture used should be one that develops acid rapidly and it is preferable that it shall have been transferred a number of times at short intervals so that it is in an active condition. Varieties that have an objectionable flavor and aroma, such as *S. lactis var. maltigenes,* yield butter cultures showing the same condition and are, accordingly, undesirable. Suitable *S. lactis* cultures can ordinarily be secured by plating out clean flavored sour milk.

The data available do not permit of any special selection of the associated organisms, although one which yields a comparatively high volatile acid seems advisable. *S. citrovorus* and *S. paracitrovorus* appear to be equally suitable and good butter cultures have also been secured by using both of them in combination with *S. lactis* but there does not seem to be any advantage in this. The value of an organism for the development of butter cultures can be told only by a trial in which it is combined with a suitable *S. lactis* organism and then carried through a series of transfers.

Associated organisms for use in developing butter cultures may be secured from various sources, such as spontaneously soured milk or cream; milk fermented after inoculation with butter showing a well developed flavor and aroma may also be used.

The butter cultures developed from the mixtures of organisms vary a great deal and the percentage that is entirely satisfactory is quite low. It seems to be difficult to get the two types to so establish themselves that the necessary changes are brought about. Often the desired flavor and aroma do not appear, due undoubtedly to the failure of the associated type to develop. In other instances coagulation may not occur or may be very slow and this presumably results from the restraining action of the associated type on *S. lactis.* Some cultures become satisfactory very quickly while others require a considerable number of transfers. Unless there is an objectionable condition or a complete lack of the desired flavor and aroma a culture should not be considered a failure until it has been through at least ten transfers in pasteurized milk. A culture that appears satisfactory should be carried for some time before a final decision is made with reference to it since a stability which makes it possible to regularly secure good results with a culture is very desirable. The use of the culture in the making of butter from high quality cream provides valuable information and when the keeping qualities of such butter are determined the test of the culture is complete.

Occasionally an associated organism is secured which regularly yields satisfactory butter cultures. At the Iowa Agricultural Experiment Station a *S. paracitrovorus* culture was used for some time that regularly gave excellent starters and was always more satisfactory than the organisms with which it was compared. It showed no other outstanding character and, since it came from a sample of off flavored cream, the source gave no suggestion of its value.

CHAPTER XIII

BACTERIOLOGY OF FERMENTED MILKS

The consumption of milk which has undergone extensive changes as a result of the growth of micro-organisms is common in all the countries in which milk is available. These products have a high food value and, in addition, some of them appear to have therapeutic properties in certain conditions, especially intestinal disorders. Their use seems to be increasing in many parts of the United States and in some localities, especially in the South, the fermented milk sold makes up a considerable percentage of the total milk sales.

There are two main types of fermentations used with the fermented milks, an acid fermentation and a combined acid and gassy fermentation, although contaminating organisms are often present and may have an influence on the changes occurring. The former occurs in various types of buttermilks and the latter in such products as kefir and kumiss.

Buttermilks. Buttermilk, in the strict sense, is the material drawn from the fat gathered in the process of buttermaking. If the cream that is churned has undergone only a clean acid fermentation the buttermilk has a flavor that is very pleasing to many people. The churning of butter on the farm from naturally soured cream that has been well cared for frequently yields a very fine flavored buttermilk, and the same is true when high quality sweet cream is delivered to creameries, pasteurized and ripened with a good butter culture. If the cream is old and has undergone a wide variety of fermentations, the flavor of the buttermilk is likely to be unpleasant and this is especially true when the cream has been treated with neutralizer. The pasteurization of cream in a sour condition usually causes the casein to harden in particles so that when the buttermilk obtained is allowed to stand these quickly settle, leaving a more or less clear whey-like material at the surface.

Because of the difficulty of securing buttermilk of a high quality from the cream delivered to many creameries, various types of products are made from skim milk, whole milk, etc. and sold under the name of culture buttermilk, artificial buttermilk or some special name adopted by the manufacturing concern. In the preparation of these buttermilks the fermentation is usually carried out with the cultures used in the butter industry so that the product is essentially butter culture. The acidity developed should not be too high, preferably about 0.75 to 0.85 per cent, although some persons seem to prefer a very acid product. After the fermentaion, the product is thoroughly agitated in a vat or churn to give it a smooth and creamy texture. Whether the buttermilk is to be made from skim milk or whole milk depends on whether or not the price for which it is to sell permits the inclusion of the fat. For many consumers the presence of fat makes the product more pleasing. When the fermentation is carried out in whole milk, the fat often forms rather firm chunks at the surface that can not be easily broken up so that a product containing fat is sometimes prepared by fermenting skim milk and adding cream after the fermentation is complete.

Culture or artificial buttermilks are objected to by many persons, especially those unfamiliar with them, because they are much thicker than natural buttermilk. By adding sweet skim milk or whole milk and thoroughly agitating, the consistency can be materially reduced. A satisfactory ratio is one part of skim milk or whole milk to two parts of the fermented milk, but this may be varied widely.

Salt is sometimes added to buttermilk to bring out the flavor. Buttermilk is occasionally modified by the addition of various materials such as sugar, lemon extract, lemon juice and beaten egg, the amounts being varied to suit the taste; other fruit juices or extracts may be employed in place of the lemon.

Bulgarian buttermilk. In some instances an artificial buttermilk containing *L. bulgaricus* is desired for special purposes, such as attempts to correct intestinal abnormalities. Cultures of *L. bulgaricus* are much more difficult to carry in pasteurized milk than butter cultures because they must be grown at from 32.2° to 43.3° C. (90° to 110° F.) and these temperatures are very favorable for the growth of the organisms resisting the pasteurization exposures. Pasteurization on two successive days at exposures such as 82.2° to 93.3° C. (180° to 200° F.) for 30 minutes commonly aids in keeping down the undesirable fermentations, as does also the selection of milk very carefully produced. Sterilized milk may be employed and is very satisfactory except for the heated flavor and the brown color produced by the high exposures necessary. The cultures of *L. bulgaricus* used for inoculating the large volumes of milk should be carried in sterilized milk to insure the inoculation of pure cultures. Milk fermented by *L. bulgaricus* does not have a flavor as pleasing to most persons as that fermented by the usual butter cultures. When the main object is to introduce *L. bulgaricus* into the intestinal tract, butter culture can be added to the milk fermented by *L. bulgaricus* and the flavor improved in this way. Attempts have been made to grow *L. bulgaricus* and the butter culture organisms together but such attempts are usually unsuccessful because of the difficulty of keeping a satisfactory balance between the types.

Cultures of *L. bulgaricus* have been employed to prevent the wheying off of buttermilk[1] secured when sour cream is pasteurized. A ropy strain of *L. bulgaricus* is used and milk fermented by it is added to the buttermilk and thoroughly distributed. The heavy body of the milk fermented by the ropy *L. bulgaricus* tends to prevent the settling of the casein in the buttermilk. The amount it is necessary to add is variable, depending on the condition of the buttermilk, the time it is to stand, etc., and should be determined by trial. The addition of butter culture also has an effect in preventing the wheying off of buttermilk and its use improves the flavor. The amount of butter culture or milk fermented by *L. bulgaricus* added is usually kept as low as possible since these products are more expensive than the buttermilk.

Bulgarian buttermilk has not given satisfactory results in many of the attempts that have been made to control the intestinal flora with it and, at present, milk fermented by *L. acidophilus,* a normal intestinal form, is much more widely used.

Acidophilus milk. Milk fermented by *L. acidophilus* is used as a buttermilk under the name of acidophilus milk. This organism is also carried in pasteurized milk with considerable difficulty; it grows comparatively slowly and the temperatures used, which are ordinarily from 35° to 37.8° C. (95° to 100° F.) are very favorable for the species resisting pasteurization. *L. acidophilus* is most commonly grown in sterile milk either in flasks or in special tanks in which milk can be heated under pressure. Careful methods of producing the milk make it possible to use lower sterilization exposures and thus to secure a product with less heated flavor and less change in color. The cultures used for inoculation must be carried under very careful conditions to insure absolute purity since a very few foreign organisms may quickly come to dominate the fermentation. Micrococci are common contaminating types, although spore formers are also encountered; the micrococci presumably are the result of contamination from the air.

---

[1] Ill. Agr. Expt. Sta. Cir. 166. 1913.

When acidophilus milk is consumed, lactose is often taken with it in an attempt to provide an abundant supply so that the growth of *L. acidophilus* will be favored. The lactose may be dissolved in the milk or in water. In some instances the establishment of the organism in the intestinal tract appears to be greatly favored by the lactose.

The results of various investigations indicate that *L. acidophilus* can be colonized in the intestinal tract much more readily than *L. bulgaricus*. The former organism appears to tolerate a lower surface tension than the latter and this may be a factor in its establishment in the intestinal tract where the surface tension is influenced by the bile salts.

Kefir. Kefir[2] is a type of fermented milk in which there is the development of both acid and gas. It apparently originated among the people living in the Caucasus Mountains and surrounding territory and is made from the milk of various animals. The fermentation may be started through the use of kefir grains which are convoluted masses made up largely of micro-organisms. These can be dried and preserved and then soaked up again when they are needed; in the dried condition they have a decidedly yellow appearance but during active growth are almost white. When added to milk the grains settle to the bottom but in the fermentation process they may be carried to the top by the gas developed. The grains increase in size during continued use and occasionally break up so that an abundant supply is provided. In certain regions the fermentation is commonly carried out in leather bags and, as the kefir is used, fresh milk is added. The bags are kept outside during the summer, either in the sun or shade depending on the temperature, but in winter they are kept in the house. When kefir is to be removed the portion wanted is frequently tied off from the remainder so as to prevent the escape of large quantities of gas when the bag is opened.

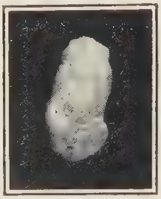


FIG. 33.—Actively growing kefir grain.

Various organisms have been isolated from kefir and it seems probable that the types present are not constant. The lactobacilli are involved in the acid development although *S. lactis* may be present also. The gas is often due to a lactose fermenting torula.

---

[2] U. S. D. A. Bul. 319. 1916.

Alcohol in variable quantities is formed along with the gas and, while it is small in amount in the freshly made product, may increase considerably with age.

A product showing an acid and gassy fermentation can be made without kefir grains. The two fermentations are best carried out separately with the acid fermentation first, although it is quite possible to run the two at the same time. The gas formation is brought about through the action of yeasts and the fermentation must be carried out in a sealed container so that the carbon dioxide produced will not be lost.

For the material in which the acid fermentation has occurred, butter culture or buttermilk may be used. This is put into bottles, about 4 per cent cane sugar added and dissolved, and then a small amount of bakers' or brewers' yeast, suspended in water, inoculated. The bottle is tightly stoppered, the cork wired in if it is not of the picnic type and the material held at about 21.1° C. (70° F.). The cane sugar is added because the yeast used does not ferment lactose and, in addition, it improves the flavor of the product for many persons. When the fermentation seems to be sufficiently advanced the milk should be cooled to check the growth of the organisms. The cooled bottles can also be handled with less danger of breakage than the uncooled because of the greater solubility of the gas at lower temperatures. It is not unusual for a bottle to be shattered due to the great gas pressure.

A product showing acid and gas can also be made from whey [3] by the general procedure outlined. There seems to be less danger of bottle breakage with whey than with milk. With the milk the casein is frequently in chunks that are rather large while, with the whey, casein is present in only negligible amounts.

Kumiss. Kumiss is a product showing an acid and gassy fermentation that is made in certain parts of Eastern Europe and Western and Central Asia. It is commonly made from the milk of the mare by inoculating with milk that has been fermented. When it is necessary to establish the fermentation anew, some fermenting or decaying material such as flesh, tendon or vegetable matter may be added to the milk. The milk of the mare is of a somewhat different composition than that of the cow, usually containing more water and sugar but less fat, protein and ash.

Yogurt, mazun, etc. In certain parts of the world milk from various animals is fermented by the inoculation of milk which con-

[3] Ia. Agr. Expt. Sta. Cir. 54. 1918.

tains essentially acid producing types. The product is called yogurt, mazun, gioddu, dadhi, leben, etc., depending on the country in which it is made. The milk is commonly boiled before it is inoculated and often the boiling may be continued until the milk is greatly concentrated; with considerable concentration the product is very thick and is often eaten with the addition of various materials such as sugar, bread and dates. The acid producing organisms are usually lactobacilli although often *S. lactis* is also present. In some instances organisms are found that play no part in the acid development.

Holding fermented milks. One of the serious problems with fermented milks is keeping them in a good condition after the fermentation is complete. With the large numbers of organisms present growth is likely to continue after the desirable stage of development has been reached and the product then deteriorates. Low temperatures provide the most satisfactory method of holding and, if the products are to be kept for any length of time, they should be held at 4.4° C. (40° F.) or lower. The quality of the fermented milks offered for sale in many places is very poor and this undoubtedly has had a pronounced effect in reducing the sales. Buttermilk is frequently served that has a yeasty and moldy flavor and aroma and is quite unsatisfactory to anyone at all familiar with a high quality product. While the acid developed in buttermilk may prevent the growth of many organisms, certain types can tolerate it and, among these, the yeasts and molds are important. The manufacture and handling of buttermilk under such conditions that yeasts and molds are not present is an aid in the putting out of a high quality product but the real solution of the holding of fermented milks is the employment of storage temperatures that are so low the growth of all organisms is materially delayed. With fermented milks in which a gassy fermentation has occurred the difficulties of holding are still greater than with buttermilks, since continued gas formation may cause the bursting of containers; it is, of course, necessary to keep the containers tightly closed in order to retain the gas which is so important in giving the product the desired flavor.

CHAPTER XIV

BACTERIOLOGY OF BUTTER

From the standpoint of the activity of micro-organisms, butter presents a very different combination of conditions than do most of the milk derivatives. The composition is such that it is not especially suited to their growth. Lactose, which is the constituent of milk most readily attacked, is present in very small amounts while the comparatively resistant fat makes up from 80 to 82 per cent. The moisture content is low and, except with unsalted butter, contains a high concentration of salt. The holding period with butter is comparatively long and, while the temperatures in retail stores and homes are not usually very low, under commercial storage conditions temperatures approximating —17.8° C. (0° F.) are commonly employed.

Determination of the number of bacteria in butter. The number of bacteria in butter is ordinarily determined by the plate method. The plating of this material is more difficult than the plating of milk or cream because of the solidification at room temperature and the failure to mix with the plating medium even when melted. The water blanks, pipettes and plates should be warmed to a temperature above the melting point of the butter; this can be conveniently done by allowing them to stand in the 37° C. (98.6° F.) incubator or the water blanks may be placed in warm water. The butter samples can be taken from a tub with a sterile trier or from a churn with a sterile spoon and, in either case, the surface should be discarded. The portion for analysis may be weighed out on a sterile paper protected by another sterile paper on the scale pan and the butter and top paper transferred to the warm water blank with sterile forceps; about one gram of butter is usually used but the amount is unimportant as long as the weight is known so that the results can be calculated to a gram basis. It is much less time-consuming and quite satisfactory to use one cubic centimeter of melted butter instead of weighing out a sample; the measuring can be readily done if the pipette is warmed in a flame and then, after delivering the butter, thoroughly rinsed out with the warm water in the blank. The butter may be conveniently melted in a sterile petri dish over a flame, although a small flask has an advantage in that it can be placed in a water bath and the temperature more carefully controlled in this way. Before removing the portion of the butter to be plated, the sample must be thoroughly shaken because of the separation of the water. After the butter is in the first water blank it is handled the same as a cubic centimeter of milk, as far as dilutions are concerned, but because the fat quickly comes to the top on standing it is necessary to work rapidly and to shake the blank just before a portion is removed. Excessive shaking, especially if the butter is not thoroughly melted, may cause the fat to form clumps and thus interfere with its distribution. The dilutions that should be used depend on the number of organisms expected from the history of the sample.

Whey agar is a very satisfactory medium for plating fresh butter in which a butter culture has been used because of the good growth the culture organisms make on it. Beef infusion agar is also satisfactory and, with old butter in which many of the organisms originally present have died, is likely to give higher counts than whey agar, due to the fact that the latter is not especially suited to the growth of micrococci and other resistant types. Beef extract agar gives a poor growth of the butter organisms. Incubation at 21° C. (69.8° F.) or room temperature for from three to five days is more satisfactory than two days at 37° C. (98.6° F.); the counts are usually higher and, due to the larger size of the colonies, the counting is more easily done.

Determination of the number of yeasts and molds in butter. A yeast and mold count on butter is ordinarily made by plating on an agar medium, using a solution of some such acid as tartaric or lactic to limit bacterial growth. Whey agar, acidified by adding one cubic centimeter of a sterile 1 per cent solution of tartaric acid crystals to each plate at the time of pouring, has been extensively used but Lund[1] prefers a wort agar to which four cubic centimeters of a sterile 5 per cent lactic acid solution are added, per 100 cc. of medium, immediately before plating; Stiritz[2] employed an agar made from malt beverage to which lactic acid was added in the proportion used by Lund and considered it more satisfactory than whey agar with tartaric acid. The acids that are used do not entirely prevent the development of bacteria but the types forming colonies that are most likely to be confused with yeast colonies are largely restrained.

---

[1] Scientific Agr. 2. P. 332. 1922.

[2] Jr. Dairy Sc. 5. P. 362. 1922.

It seems to be impossible to add enough acid to prevent bacterial growth completely without interfering with the growth of some of the yeasts and molds. Whey agar, especially, seems to permit considerable bacterial development.

Because of the small number of yeasts and molds sometimes present in butter one cubic centimeter is commonly used in plates but, with a product that may show high counts, plates should also be poured with 1/10, 1/100 and sometimes with 1/1,000 cubic centimeter amounts. The dilutions may be secured by putting either one cc. or ten cc. of butter through a series of warmed water blanks, using warmed pipettes. If only general information on whether the counts are high or low is desired this can be secured from plates in which one cc. of butter is used. The uniform mixing of one cc. of butter with the plating medium is rather difficult and large masses of fat are often present in the poured plates; these masses undoubtedly tend to keep the yeast and mold count lower than it should be by enclosing yeast and mold cells and thus preventing their development into colonies.

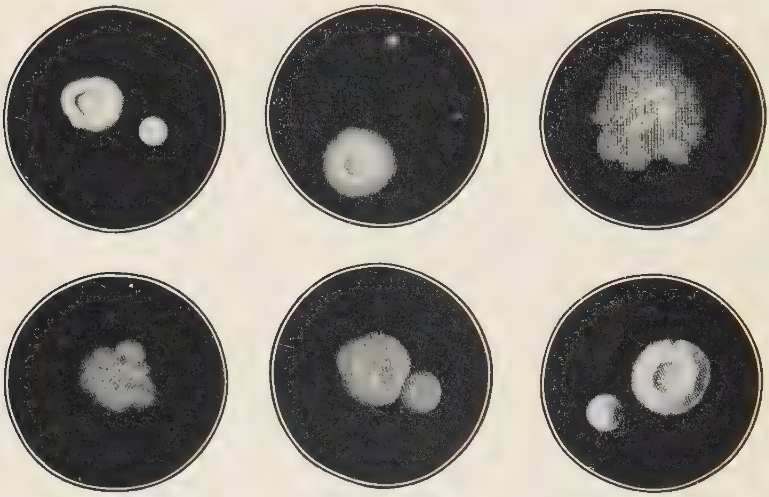


Fig. 34.—Various types of yeast colonies on plates poured for yeast and mold counts on butter.

Plates for yeast and mold counts may be incubated at room temperature for from two to five days, depending on the growth of the organisms, or a constant temperature such as 21° C. (69.8° F.), 25° C.

(77° F.) or 30° C. (86° F.) may be used. When large numbers of molds are present it is especially necessary that the plates be watched carefully and the counting done before the mold growth becomes so heavy that recognition of the individual colonies is difficult.

The yeasts and molds may be enumerated together and the results expressed as the yeast and mold count or the two groups of organisms may be counted separately; with surface colonies the organisms are easily differentiated but with sub-surface colonies more difficulty is encountered.

Redfield[3] has proposed a method of counting yeasts and oidia in butter with an adaptation of the Breed method for counting bacteria in milk. The butter is melted at 45° C. (113° F.) and allowed to stand at this temperature, or slightly lower, until the fat separates. The water and materials other than fat are mixed by filling and emptying a pipette, after which 0.01 cc. is spread on a glass slide, care being taken to exclude the fat; the area covered is one, two or four square centimeters, depending on the number of yeasts and oidia expected, and if necessary to obtain uniform smears, distilled water is used for dilution. The slide is dried, stained and examined in the usual way. This method gives the results per cubic centimeter of the mixture of moisture, curd, etc. and includes both living and dead organisms so that the results are not comparable with those secured by the plate method.

Johns[4] has adapted the Frost little plate method to the determination of the yeasts and molds in butter. An incubation period of 12 to 18 hours at 25° C. (77° F.) was found the most suitable; when a shorter period was used, it appeared advisable to raise the temperature to 30° C. (86° F.) while, with a longer period, a lower temperature was desirable. A study of the method led to the conclusion that it furnished a reasonably accurate indication of the mold content of butter when this was above 100 per cc., as determined by the usual plate method; on the average the mold counts obtained were twice as large as those secured with the usual method. The yeast counts showed greater variations from the values obtained with the usual method than did the mold counts but a satisfactory relationship between the results of the two methods was secured in 74.6 per cent of the samples analyzed.

Sources of the organisms in butter. Bacteria, yeasts and molds get into butter from various sources, such as the cream, the equipment, the air and the persons making and handling the product. The number of organisms in the cream at the time it goes to the churn largely determines the number that will be present in the butter, although the churn is a difficult piece of equipment in which to control the growth of micro-organisms and large numbers may be added from this source.

---

[3] Jr. Dairy Sc. 5. P. 14. 1922.

[4] Scientific Agr. 8. P. 353. 1928.

Numbers of micro-organisms in fresh butter. The bacterial content of fresh butter is extremely variable and depends primarily on the method of manufacture. Although sweet cream regularly contains fewer organisms than sour cream, pasteurization is very efficient with the latter and largely eliminates the original difference so that the most important factor causing a high bacterial content with fresh butter is the use of a butter culture. When such a culture is employed, either with or without actual ripening of the cream, the numbers of bacteria in the cream and in the butter made from it are greatly increased. There may be bacteria added to the cream or butter as a result of contamination in the plant but the number coming from this source is small compared to the number coming from the butter culture. The bacterial content of fresh butter varies from a few thousand per cubic centimeter in the case of a product carefully made from thoroughly pasteurized cream without the use of a culture, to several million when a culture is employed.

The numbers of yeasts and molds in butter also vary widely. Some samples may fail to show any of these forms in one cubic centimeter while, with others, the counts run into the hundreds of thousands. Ordinarily when the yeast and mold count is high the yeasts are more numerous than the molds.

Significance of the micro-organisms in butter. The number of bacteria in butter is of no significance because of the high counts expected with the usual methods of manufacture. Butter is typically a product in which the desired condition is brought about through the action of micro-organisms. The bacterial content of butter may be of interest in connection with the study of deterioration. The types of bacteria present are sometimes very evidently foreign to a product carefully made from high quality cream and are the result of contamination from the equipment or the presence of resistant organisms in the cream.

The number of yeasts and molds in butter is an indication of the care used in manufacture. A low count shows that the yeasts and molds that were in the cream have been very largely destroyed by pasteurization and that the plant operations have been carried out in such a manner as to prevent the addition of significant numbers. A high count is due to inefficient pasteurization or contamination in the plant, usually from equipment that is poorly cared for. Some plants have yeast and mold counts on the butter under five per cubic centimeter for extended periods while others have excessive counts. It is difficult to set a standard that can be accepted as satisfactory but a plant should aim to reduce the count through improved methods until small numbers are regularly obtained.

The raw cream coming to butter plants often contains very large numbers of yeasts and molds, with the yeasts ordinarily more numerous than the molds. In a number of samples of raw sweet cream North and Reddish [5] found a maximum of 75,000 yeasts per cc. with the microscopic method and a maximum of 5,200 with the plate method; oidia were not detected by the microscopic method, while the plate method gave a maximum of 500 per cc. Redfield,[6] using the microscopic method, investigated the cream coming into a centralizing plant and found that it commonly contained more than 1,000,000 yeasts per cc.; the number of oidia was lower but frequently ran into the hundreds of thousands. These results show the possibilities of cream as a source of yeasts and molds in butter.

Although yeasts and molds may be added to cream from various pieces of equipment, the churn appears to be especially important in this connection. The contamination from the churn can be measured by adding sterile water and then, after agitation, plating it out. Lund [7] has reported the following results which illustrate the contamination from a churn.

1. Butter made in a new churn from properly pasteurized cream usually has a low yeast count.
2. Where two or more churnings a day are made in the same churn, butter from the first churning will almost invariably contain the largest number of yeasts.
3. If a churn stands idle for several days the yeast content of butter made in it when it is brought into use again will usually be high.
4. Repacking the boxes where the worker roller pins pass through the head of the churn frequently results in a reduction of the yeast content of the butter, sometimes to a marked degree.

---

[5] Jr. Dairy Sc. 4. P. 510. 1921.

[6] See ref. 3.

[7] See ref. 1.

Stiritz[8] found that cream may be contaminated with yeasts and molds from butter culture but that this source has little effect on the yeast and mold content of butter, except in rare cases when an extremely inferior quality of culture is used.

The excessive numbers of yeasts found in certain samples of butter may be due to growth. North and Reddish[9] noted some large increases in the numbers of yeasts in butter during a period which involved from one to seven days at 4.4° to 7.2° C. (40° to 45° F.), transportation by express and then from one to three days at —11.1° C. (12° F.); during the same period no increase in oidia was noted.

The relationship of the composition of butter to the yeast and mold content has been investigated by Macy,[10] using results secured on 2,700 lots of butter shipped to the eastern markets. As the salt content of the butter increased the percentage of high counts decreased; this relationship was more closely correlated with the molds than with the yeasts which suggests that the molds are the more sensitive to salt.

Types of micro-organisms in fresh butter. The principal bacteria in fresh butter made with butter culture are the normal culture organisms. The other species present have either survived the pasteurization or gotten in from various plant sources and are, accordingly, of a variety of types. Because of the great predominance of the culture organisms, plates which show colonies in numbers that are satisfactory for counting may contain only these species and, to secure the others, plates must be poured with larger quantities of butter. The yeasts found are of various types, none of which tend to predominate. *Oidum lactis* is the mold most commonly noted and, in many butter samples showing high mold counts, it is the only type present.

Comparison of the bacterial contents of cream and of the butter made from it. When cream is churned the great majority of the organisms present are held in the buttermilk and the fresh butter contains a much smaller number of bacteria per cubic centimeter than the cream from which it came. This relationship is shown in the following summary of the results reported by Grimes[11] for cream in which butter culture was used:

---

[8] See ref. 2.

[9] See ref. 5.

[10] Jr. Dairy Sc. 10. P. 384. 1927.

[11] Jr. Dairy Science. 6. P. 427. 1923.

Comparison of the Numbers of Bacteria in Cream and in the Buttermilk and Butter Secured from It

Type of Cream	Number of Trials	Average Acidity of Cream, Per Cent	Average Number of Bacteria per CC.		
			Cream at Churning	Buttermilk	Butter
Pasteurized sweet cream plus 10 per cent starter	8	0.28	278,000,000	336,000,000	4,100,000
Pasteurized sweet cream ripened to serum ×0.0063............	22	0.44	196,000,000	211,000,000	2,813,000
Pasteurized sweet cream, highly ripened.......	7	0.55	232,000,000	282,000,000	2,650,000

The average bacterial content of the buttermilk was considerably higher than that of the cream; this may have been influenced by the breaking up of clumps or chains of organisms and by actual growth but was undoubtedly due, in part, to the failure of the butter to carry any considerable number of organisms with it. Grimes concluded that butter made from cream in which butter culture was used retained, per cubic centimeter, from about 0.5 to about 2 per cent of the bacteria per cubic centimeter of the cream, generally about 1 per cent. With butter made from sweet cream this investigator found that the butter retained, per cubic centimeter, from about 5 to about 30 per cent of the bacteria per cubic centimeter of the cream.

Pasteurization of sour cream. The pasteurization of sour cream presents a different problem than the pasteurization of sweet milk or cream because of the large number of organisms contained. A high bacterial efficiency is regularly secured. The organisms in sour cream are mainly the result of growth and, since the conditions in cream are especially satisfactory for *S. lactis,* this type makes up a considerable percentage of the bacteria; it is easily killed by heat and, accordingly, a high percentage of the total organisms are destroyed. The bacterial content of pasteurized sour cream varies widely, due to variations in the numbers of resistant organisms in the raw material.

The effect of different pasteurization exposures on the bacteria in sour cream has been studied by Hammer,[12] the organisms being determined by plating on beef extract agar and incubating the plates

[12] Ia. Agr. Expt. Sta. Bul. 156. Part 2. 1914.

at 37° C. (98.6° F.) for 48 hours; representative data showing the bacterial counts before and after pasteurization and the bacterial efficiencies are as follows:

Destruction of Bacteria during the Pasteurization of Sour Cream

Method of Pasteurization	Acidity of Cream, Per Cent	Exposure Used	Bacteria per CC.		Bacterial efficiency
			Raw Cream	Pasteurized Cream	
Holding. .	0.48	63.3° C. (146° F.), 20 min.	550,000,000	87,000	99.984
Holding. .	0.48	62.8° C. (145° F.), 20 min.	375,000,000	38,000	99.990
Holding. .	0.62	62.8° C., 25 min.	376,000,000	30,850	99.992
Holding. .	0.54	62.8° C., 25 min.	515,000,000	255,000	99.950
Holding. .	0.66	62.8° C., 20 min.	390,500,000	21,600	99.994
Flash. . . .	0.47	82.2° C. (180° F.)	254,500,000	5,450	99.998
Flash. . . .	0.56	82.2° C.	352,000,000	2,750	99.999
Flash. . . .	0.44	82.2° C.	620,000,000	3,100	99.999
Flash. . . .	0.42	82.2° C.	820,000,000	17,650	99.998

In these results flash pasteurization at 82.2° C. was more efficient than the holding method in destroying the organisms in sour cream.

Hunziker, Spitzer, Mills and Switzer[13] have reported studies on the destruction of organisms during the pasteurization of sour cream. Thirty-three lots of cream were examined when raw and again after pasteurization by each of three methods. The total counts were determined with a beef extract agar, the acid formers with the same medium except that litmus was added, the liquefiers with a beef extract gelatin and the yeasts and molds with an acidified beef extract whey agar; the agar plates were incubated at 35° C. (95° F.) for four days and the gelatin plates at 21° C. (69.8° F.) for five days The summarized results are presented in the table on page 363. These data show a higher destruction of organisms with the holding method than with the flash method at 85° C. and in this respect differ from those secured by Hammer; flash pasteurization at 73.9° C. was relatively inefficient. The data from the two sources agree in showing that a high destruction of the organisms in sour cream is secured with the holding method or with the flash method at either 82.2° or 85° C.

---

[13] Ind. Agr. Expt. Sta. Bul. 208. 1917.

Destruction of Bacteria during the Pasteurization of Sour Cream

	Organisms per CC.			
		In Pasteurized Cream		
	In Raw Cream	62.8° C. (145° F.) 20 Min.	73.9° C. (165° F.) Flash	85° C. (185° F.) Flash
Total count	209,714,285	113,574	14,768,000	1,416,029
Acid formers	123,985,714	42,928	8,415,000	837,352
Liquefiers	16,182,854	3,035	1,295,000	81,429
Yeasts and molds	4,032,000	2,201	197,911	16,782

	Per Cent Decrease Due to Pasteurization		
	62.8° C. (145° F.) 20 Min.	73.9° C. (165° F.) Flash	85° C. (185° F.) Flash
Total counts	99.85	89.82	98.97
Acid formers	99.95	89.77	96.07
Liquefiers	99.98	90.19	99.63
Yeasts and molds	99.88	86.18	98.90

There is evidence that certain bacteria are less resistant to heat in the presence of acid than in its absence. Hammer and Trout[14] noted that 12 cultures of yellow cocci survived 75° C. (167° F.) for 30 minutes in normal milk while in milk in which *S. lactis* had produced sufficient acid to cause coagulation only 1 survived 65° C. (149° F.) for 30 minutes and all were killed at 70° C. (158° F.) for 30 minutes. Unpublished results obtained at the Iowa Agricultural Experiment Station show a decrease in the heat resistance of *S. lactis* in the presence of acid; a small amount of a coagulated culture highly diluted with sweet milk was more resistant than the culture direct, although the number of organisms per cubic centimeter was much less.

Changes in the number of bacteria in butter. An increase in the number of bacteria in butter may occur under certain conditions but the usual change is a decrease, which is especially rapid during the early part of the holding period. The decrease is due primarily to the death of the butter culture organisms; these find conditions unsatisfactory and die out rather quickly. It appears that while certain species may occasionally multiply in butter, others never do.

---

[14] Jr. Dairy Sc. 11. P. 18. 1928.

One of the factors especially important in connection with the multiplication of bacteria in butter is the temperature of holding. Growth is more likely to occur at room temperature or 10° C. (50° F.) than at 0° C. (32° F.) or lower and under commercial storage conditions multiplication probably never occurs. In general, the salt in butter has a restraining influence on bacteria and multiplication in unsalted butter is more common than in salted; when bacterial increases occur with the usual salting they are less rapid than in unsalted butter under the same conditions and the maximum reached is lower. Washburn and Dahlberg[15] noted that in unsalted butter the bacteria increased more rapidly at 14.4° C. (58° F.) than in salted butter but at —26.1° C. (—15° F.) they decreased more rapidly. Data secured at the Iowa Agricultural Experiment Station suggest that the type of cream churned may be a factor in the growth of organisms in butter. A product made from sweet cream appeared to be more suitable for growth than one made from ripened cream. Such an influence may be due to the general inhibiting effect of the products of bacterial growth. In some instances the growth of bacteria in butter is more rapid at the surface than in the interior, due presumably to the air supply.

The extent of the decrease in the number of bacteria in butter and the influence of the type of butter on this are illustrated by data secured by Grimes,[16] who found that during six months storage at —21.1° C. (—6° F.) from 95 to 99 per cent of the bacteria in butter made with butter culture died off while the average decrease in butter made from pasteurized sweet cream was approximately 20 per cent and the maximum 65 per cent.

Types of micro-organisms in old butter. The types of micro-organisms in old butter are extremely variable and, in general, are those that can resist unfavorable conditions. Micrococci are often conspicuous and occasionally spore forming bacteria are found. Yeasts and molds may also be encountered. With old butter, the content of micro-organisms is usually so low that types which are prominent on plates showing a satisfactory distribution of colonies are present in comparatively small numbers per cubic centimeter.

[15] Jr. Dairy Sc. 1. P. 114. 1917.

[16] See ref. 11.

SOURCES OF THE FLAVOR IN BUTTER

General flavor of butter. The general flavor of butter is a very important character from the standpoint of its market value and the one which is most difficult to control. Many lots of butter are considered perfect in body, color, salt and package but this is never the case with the flavor. The general flavor is made up of flavors from a number of sources and what the butter judge evaluates is, in reality, a combination of flavors. These may be divided into two groups, those that are desirable and those that are undesirable.

Desirable flavors of butter. The desirable flavors of butter come from two main sources, (1) the fat and (2) the products formed by certain bacteria.

The flavor of the fat of milk is more or less characteristic and is not found with other edible fats, such as those used in the manufacture of margarine. Butter churned from cream in which there has been no development of micro-organisms has a definite butter taste that is very pleasing, but it is lacking in the flavor that is regarded as very necessary in a high grade product and which is found only in butter made so as to contain the products of the growth of desirable bacteria. These products may be secured through natural souring but, with this procedure, objectionable flavors occur so regularly that under commercial conditions cultures of organisms known to give a desirable flavor are commonly employed. These organisms may be grown in the cream during a ripening period or they may be grown in milk which is then added to the cream and the mixture held at a low temperature. By preceding the use of the cultures with the pasteurization of the cream, the field is very largely left clear for the desirable bacteria.

Undesirable flavors in butter. While the development of the desirable bacterial products in the proper proportion is a difficult problem in the manufacture of butter, the prevention of the undesirable flavors is even more difficult. Conditions may be ideal for the activity of the bacteria producing desirable flavors but, if undesirable flavors are present or appear, the quality of the product is seriously influenced.

The undesirable flavors present in butter may be divided into two main groups from the standpoint of the time of their appearance, (1) those present in fresh butter and (2) those that develop on holding. Various factors may be responsible for these flavors but the products of the growth of micro-organisms, either in the cream or in the butter, are directly or indirectly concerned in the development of a considerable number of them.

The amounts of various materials required to influence the flavor and aroma of butter are extremely small, which indicates that the changes resulting in their development must also be slight. The difficulty of determining the nature of these slight changes greatly complicates the study of the whole problem of butter deterioration. In many instances pronounced off flavors appear when various chemical examinations show nothing abnormal.

Causes of undesirable flavors in fresh butter. The flavors present in fresh butter are closely related to the flavors present in the cream as it goes to the churn. Certain flavors are more pronounced in the butter than in the cream, through their association with the fat, while others are less pronounced because they are chiefly associated with the non-fatty constituents and are largely carried away with the buttermilk. The washing of the butter may also tend to decrease the intensity of certain flavors.

The causes of undesirable flavors in the cream coming to butter plants are essentially the same as those responsible for undesirable flavors in milk and include the growth of micro-organisms, the condition of the producing animals, the materials consumed by them, especially the feeds, the absorption of odors and the addition of foreign substances. The growth of micro-organisms is the most important of these and the flavors developed in this way tend to cover up the flavors due to other causes, unless the latter are very pronounced. However, with cream that is kept cool and delivered frequently so that the multiplication of micro-organisms is controlled, the other causes may be of considerable significance.

The undesirable flavors present in cream in which there has been extensive growth of micro-organisms are of a variety of types, some of which are very definite while others are indefinite and difficult to describe. Among the more definite of such flavors are malty, yeasty, bitter, cheesy, musty, metallic and fishy flavors. Each of these is due to one or more types of organisms which find conditions for growth very favorable. Sometimes two of the flavors are present in cream at the same time. The objectionable flavors of sweet cream are such absorbed flavors as fly spray, gasoline, kerosene and barny, such feed flavors as onion, rape, beet top, sweet clover and silage and flavors due to bacterial action.

Incorrect methods in the plant operations may cause undesirable flavors in the cream that are carried over into the butter. Carelessness in the heating during pasteurization so that portions of the cream reach excessive temperatures sometimes gives a heated flavor that is pronounced enough to be objectionable. Improper neutralization of cream, especially over neutralization, may cause a definite undesirable flavor in the butter made from it. Flavor defects in the butter cultures are commonly carried over into the butter with which the cultures are used.

Causes of undesirable flavors that develop in butter. Although the flavor of butter may improve for a short period after its manufacture through the decrease of some objectionable condition, such as a heated flavor, or through an intensifying of the desirable flavor and aroma, the general effect of holding is a decrease in the quality. The extent of this decrease varies greatly; with butter properly made from high quality cream and stored at suitable temperatures it may be negligible while, with a poorly made product stored under unfavorable conditions, it may be very serious.

The changes in butter which result in the development of undesirable flavors probably involve bacterial, chemical and enzymatic action. Changes which are in themselves strictly chemical may be greatly influenced by the products of the growth of micro-organisms, especially acids, so that micro-organisms play a role in butter deterioration, both directly and indirectly.

In considering the flavors that develop in butter during holding, the ability of this product to absorb odors must be recognized. Because of this absorbing power butter should be kept away from materials having pronounced odors, such as fish, smoked meats, melons, cucumbers, etc. Butter of an excellent quality often acquires a very objectionable flavor by being held in an ice box containing other food products; such an exposure is especially serious after the package has been opened. Under plant conditions the odor of a cooler in which butter is held is of importance in this connection, especially when the product is exposed in boxes or trays.

FACTORS INVOLVED IN BUTTER DETERIORATION

The differentiation of bacterial, chemical and enzymatic changes in butter deterioration is very difficult. The temperature and general conditions that favor the development of bacteria also favor the action of enzymes and some of them favor chemical action. Reagents, such as chloroform, can be used to prevent the growth of organisms but changes that take place can still be due to either enzymatic or chemical action; moreover, the presence of chloroform seriously interferes with the judging of the butter.

The study of butter deterioration is complicated by the necessity of basing the conclusions as to the extent of the change on the senses of taste and smell, rather than on accurate chemical measurements. It is extremely difficult to keep exactly the same standard in judging a lot of butter on widely separated days and, accordingly, the extent of the deterioration may be incorrectly rated.

Bacterial action in butter deterioration. The growth of micro-organisms which sometimes occurs in butter, especially at favorable temperatures, may result in the development of undesirable flavors. The general conditions under which such growth takes place indicate that the direct action of micro-organisms may be important in butter deterioration at room or ice box temperature and with certain lots, particularly unsalted butter, may occur at somewhat lower temperatures. Under commercial storage conditions the growth of micro-organisms in butter is very unlikely and the deterioration is due to other causes.

The changes brought about by the direct action of micro-organisms are not confined to any one of the butter constituents but apparently involve the comparatively resistant fat as well as the protein and small amounts of lactose present. The species that are active are rather limited in number because many organisms find conditions in butter entirely unsatisfactory for growth.

It appears that at one time there was a definite tendency to ascribe all butter deterioration to the action of bacteria. This may have been due to their recognized importance in bringing about changes in milk and cream, but the infrequency of low temperature storage, so that the observations were largely limited to higher temperatures, was also a factor. Evidence gradually accumulated which suggested that butter often deteriorates under such conditions that bacterial action is improbable. Experimental results also showed that butter could deteriorate at fairly high temperatures without an active increase in organisms; for example, Rogers[17] noted that canned butter stored at room temperature developed off flavors during a period when the numbers of micro-organisms, as determined by both aerobic and anaerobic lactose gelatin plates, were decreasing.

Data have been presented from various sources which indicate that the addition of organisms to cream to be made into butter has little influence on the keeping quality of the product when held at low temperatures. Rogers, Berg, Potteiger and Davis[18] compared three types of butter, (1) from raw cream, (2) from pasteurized cream, (3) from pasteurized cream inoculated and held so that after 24 hours the numbers and types of the organisms approximated those before pasteurization without an appreciable increase in acidity having occurred, and found that the reinoculation of the cream had little or no effect on the keeping quality. Grimes[19] reported that certain proteolytic bacteria, various yeasts and *O. lactis* were added individually to both ripened and unripened cream without the keeping qualities of the butter being significantly influenced during storage for six months at —21.1° C. (—6° F.); mixtures of *O. lactis* and various yeasts gave the same general result.

---

[17] U. S. D. A. B. A. I. Bul. 57. 1904.

Chemical action in butter deterioration. The inadequacy of the action of micro-organisms as an explanation of butter deterioration under all conditions gradually led to the conclusion that chemical changes are important. Such changes undoubtedly occur at the various temperatures to which butter is subjected; under storage conditions they may be the principal cause of deterioration while at higher temperatures, although actually more extensive, they may be relatively less important because of the influence of other factors. It appears that the chemical changes are of various types and that the general conditions may largely determine which type is of the greatest significance.

Influence of air in butter deterioration. The relationship of air to butter deterioration has long been suspected because of the frequency with which the surface portion of a package of butter shows a more undesirable flavor than the inner portion. It should be noted that air tends to favor the growth of certain micro-organisms in butter and may influence deterioration in this way but such an effect would not be operative at very low temperatures and chemical action is indicated. Gray[20] found that under various conditions of storage, including storage at —23.3° C. (—10° F.), butter in full cans kept much better than in cans partially full, and ascribed this to the presence of air in the unfilled cans. Dyer[21] studied the oxidation in butter by analyzing the gas present after varying periods of holding; trials were carried out with butter made in various ways and kept at different temperatures. He concluded that the production of off flavors so commonly met with in cold storage butter is attributable to a chemical change, expressed through a slow oxidation, progressive in some one or more of the non-fatty constituents. The extent of the chemical change was proportional to the quantity of acid present in the cream from which the butter was made; it was very slight in butter made from sweet cream and held 180 days at —17.8° C. (0° F.), only a little greater in butter made from cream containing 0.25 per cent acid as a result of the addition of 15 per cent of butter culture and held for 205 days at —17.8° C. but very pronounced in butter made from cream containing 0.71 per cent acid, due to the addition of commercial lactic acid, and held 202 days at —17.8° C. With fat alone the oxidation was slight while with buttermilk it was rapid, when acid was present, but slight in the sweet product. The quantity of carbon dioxide in cold storage butter appeared to have a certain relation to the quantity of buttermilk in the butter; during storage this quantity sometimes increased and then decreased.

---

[18] U. S. D. A. B. A. I. Bul. 162. 1913.

[19] See ref. 11.

[20] U. S. D. A. B. A. I. Bul. 84. 1906.

[21] Jr. Agr. Res. 6. P. 927. 1916.

Influence of acid on butter deterioration. At one time it was commonly believed that the development of acid in cream intended for churning gave the butter more satisfactory keeping qualities. This idea presumably resulted from the ease with which acid foods can be preserved and ignored the early experimental data. In 1889, Conn[22] questioned the superior keeping quality of ripened cream butter. Patrick[23] reported one comparison of sweet and ripened cream butter in which the former appeared to have slightly better keeping qualities. Patrick, Leighton and Bisbee[24] and later Patrick, Leighton and Heileman[25] carried out trials and found that the sweet cream butter deteriorated less than that made from ripened cream. These early experiments are of special interest because of the comparatively high storage temperature, approximately 10° C. (50° F.), used in most of the trials. Gray[26] noted that butter made from cream that was sweet and in a good condition kept well when stored at either —23.3° C. (—10° F.) or —12.2° C. (10° F.) and also after removal from storage, while butter made from cream that was sour and in a fair condition kept well at these temperatures but deteriorated rapidly after removal from storage; butter culture was used with both types of cream. In a study of fishy butter Rogers[27] found that in all cases where the records were complete the experimental butter which became fishy was made from high acid cream and that fishiness developed in butter made from cream acidified with lactic or acetic acid; cream with a high acidity did not uniformly develop fishiness, however. This investigator stated that, in his opinion, fishy flavor is caused by a slow spontaneous chemical change to which acid is essential and which is favored by the presence of small amounts of oxygen. Rogers and Gray[28] concluded that butter frequently undergoes marked changes, even when stored at very low temperatures, and that these changes are more marked as the acidity of the cream from which the butter is made is increased; changes in the high acid butter were not checked by heating the ripened cream. Rogers, Thompson and Keithley[29] compared the deterioration in butter made from unpasteurized ripened cream with that made from pasteurized sweet cream, the two types of butter being made in different creameries. The former had an average score of 94.20 when fresh and 87.25 after eight months' storage while the latter had an average score of 94.69 when fresh and 92.33 after storage; the judges found fishiness very common in the unpasteurized ripened cream butter while it was rare in the pasteurized sweet cream product. Additional comparisons by these investigators gave similar results.

---

[22] Conn. (Storrs) Agr. Expt. Sta. 2nd An. Rpt. P. 63. 1889.

[23] Ia. Agr. Expt. Sta. Buls. 8 and 11. 1890.

[24] Ia. Agr. Expt. Sta. Bul. 18. 1892.

[25] Ia. Agr. Expt. Sta. Bul. 21. 1893.

[26] See ref. 20.

Butter made from sweet cream lacks the pleasing flavor and aroma given by the butter culture organisms and, accordingly, attempts have been made to get both the desired flavor and aroma and good keeping qualities by the use of low acidities in the cream. Mortensen[30] secured data on butter made (1) from sweet cream, (2) from sweet cream plus culture and (3) from ripened cream. In general, the ripened cream butter scored higher than the other types when fresh but deteriorated more rapidly so that at the end of the nine months' storage period the score was lower. The sweet cream butter scored lower than the other types when fresh but, because of the slow deterioration, scored higher after long holding. The butter made from sweet cream to which culture was added kept better than the ripened cream butter, and both when fresh and after holding two months, scored higher than the sweet cream butter. White[31] reported

[27] U. S. D. A. B. A. I. Cir. 146. 1909.

[28] U. S. D. A. B. A. I. Bul. 114. 1909.

[29] U. S. D. A. B. A. I. Bul. 148. 1912.

[30] Ia. Agr. Expt. Sta. Bul. 207. 1922.

[31] Dairy Produce 34. P. 24. Jan. 3, 1928.

results showing the deterioration of butter made from sweet cream and from cream with various acidities, the lower acidities being secured by the addition of culture while, for the higher acidities, the cream was ripened. All of the cream was pasteurized at 62.8° C. (145° F.) for 30 minutes; in some cases pasteurization was carried out after adding the culture, but this did not appear to have any material influence on the deterioration of the butter. The following table, representing 125 churnings, gives the deterioration of the butter during storage at —17.8° C. (0° F.) for eight months.

Influence of the Acidity of the Cream on the Deterioration of Butter

Acidity of Cream When Churned, Per Cent	Score of Butter		Decrease in Score during Storage
	When Fresh	After Storage	
0.15	91.02	90.17	.85
0.25	91.09	90.14	.95
0.31	91.02	89.83	1.19
0.35	91.23	88.73	2.50
0.45	90.94	87.39	3.55

These results show very little deterioration in the butter made from cream having 0.15 or 0.25 per cent acid; when the cream had 0.31 per cent acid the deterioration was slightly greater and when it had 0.35 per cent or more acid the deterioration was pronounced. The data accordingly suggest that, when the butter is to be stored for eight months at low temperatures, acidities up to 0.31 per cent give comparatively little deterioration; other results presented by White indicate that, if the storage is under less favorable conditions, there may be considerable deterioration with an acidity as high as this.

The keeping qualities of butter made with culture but with only a low acidity in the cream are well illustrated by the data secured in connection with the scoring contests held by the National Creamery Buttermakers' Association. In 1924,[32] out of 307 tubs of butter, 68 were made from sweet cream plus culture while 48 were made from sweet cream without culture. The 68 tubs made with culture had an average score of 93.44 when going into storage and 92.90 on coming out, while the 48 tubs made without culture averaged 92.51 when going in and 92.03 on coming out. Accordingly, the butter made with culture scored 0.93 higher when going into storage and 0.87 higher on coming out. White[33] has tabulated the combined results of the 1925 and 1926 contests, on the basis of the acidity of the cream when churned; the only entries considered were those for which the desired data were available and which were made from cream containing 0.2 per cent acid or less when received. The butter was stored for about five months in 1926 and somewhat less in 1925. The tabulation given by White is as follows:

---

[32] Dairy Record. 34. P. 10. Nov. 26, 1924.

NATIONAL CREAMERY BUTTERMAKERS' ASSOCIATION COLD STORAGE BUTTER CONTESTS–1925–1926. (292 ENTRIES)

Number of Samples	Acidity of Cream, per cent	Average Acidity Received	Average Acidity when Churned	Average Score of Butter		Points Lost During Storage	Showing No Decrease in Score During Storage		Scoring 94 or Higher After Storage	
				Fresh	After Storage		Number	Per cent	Number	Per cent
60	0.20 or less—no culture..	0.169	0.172	92.83	92.60	0.23	24	40.0	3	5.0
74	0.20 or less—culture used.	0.153	0.186	93.28	92.77	0.51	20	27.0	7	9.5
77	0.21 to 0.25............	0.167	0.231	93.16	92.52	0.64	22	28.6	6	7.7
52	0.26 to 0.30............	0.165	0.287	93.43	92.59	0.84	10	19.2	4	7.7
17	0.31 to 0.35............	0.182	0.331	93.11	92.08	1.03	3	17.6	0	0.0
12	0.36 to 0.45............	0.164	0.401	93.21	91.45	1.76	2	16.6	0	0.0

An increase in the deterioration of the butter with an increase in the acidity of the cream is very evident in these results.

The use of butter cultures in such a way that the cream will have a low acidity at churning is now commonly practiced with the object of getting considerable flavor and aroma in the butter and also good keeping qualities. The excessive acidities at one time developed in cream are regularly avoided because of their recognized relationship to deterioration. The acidity may be varied to a certain extent, depending on the period the butter is to be held, but is always low compared to the acidities at one time employed. Very often the temperature of the cream and added butter culture prevents the growth of the organisms and the products of bacterial growth that give a desired flavor to the butter come entirely from the butter culture. When actual ripening is carried out, the period is kept so short that only a small amount of acid is developed.

The deterioration, which so commonly occurs with butter made from cream containing considerable acid, is not of a definite type but includes the development of various off flavors. At low holding temperatures a rather characteristic storage flavor often appears first and is then followed by other flavors which become more and more objectionable as the butter ages. In some cases one type of off flavor follows another in a rather definite sequence.

---

[33] See ref. 31.

Acid influences the deterioration of butter primarily through its effect in increasing the rate of chemical changes. Although acid may tend to favor the growth of some of the yeasts and molds under certain conditions, no such an effect can be expected in butter held in storage. Since the acid present in cream is due to the activity of bacteria, its effect on the deterioration of butter is an indirect result of bacterial development and, in the final analysis, the influence of acid is a bacteriological problem.

Influence of the neutralization of cream on butter. The neutralization of cream may influence, in various ways, the flavor and aroma of the butter made from it. Certain objectionable products formed during the fermentation of the cream, for example butyric acid, are fixed as salts and, in this condition, are much less important as a cause of off flavors and aromas; moreover, the salts are quite readily carried away in the buttermilk and wash water. Reducing the acidity of the cream also has a very pronounced influence in limiting the development of off flavors during the holding of the butter and neutralization, in combination with pasteurization, thus makes it possible to manufacture butter with very good keeping qualities from cream in which there has been extensive changes due to microorganisms.

Influence of metals in butter deterioration. The relationship of absorbed metals to butter deterioration is suggested by the results of various investigators. Rogers, Berg, Potteiger and Davis [34] found that the addition of iron to cream, even in as small an amount as one or two parts per million parts of cream, had an influence on the flavor of the butter; there was no evidence that the nature of the flavor was appreciably changed but the rate of development was accelerated. The action of copper was similar but perhaps more intense. In general, only a relatively small part of the iron goes into butter as compared with the buttermilk and, in accordance with this, the presence of a flavor due to iron was most noticeable in the latter product. A large percentage of the iron retained by the butter was present in the curd. These investigators also found that a strong odor could be produced in milk by the addition of small amounts of iron salts, the ferrous salts being more active than the ferric. Hunziker and Hosman [35] reported that direct contact of butter with either iron or copper developed a tallowy flavor and aroma and turned the butter white; the action started where the butter was exposed to the air and gradually went through the entire mass. Copper apparently caused a more rapid and more intense action than the iron. In trials carried out by Palmer and Combs [36] tallowiness was produced in butter by the addition of 0.017 per cent copper lactate to either raw cream or cream which had been pasteurized at 79° to 80° C. (174.2° to 176° F.) for 15 minutes. In each of three experiments, typical tallowiness and bleaching occurred in the raw cream butter before it appeared in the pasteurized cream product.

---

[34] See ref. 18.

During the handling of milk and cream there is ordinarily an opportunity for the acquisition of small amounts of metal from the utensils and equipment. This may occur through the mechanical removal of particles but the formation of salts and electrolytic action are more serious. The formation of salts is influenced by the development of acid but some of the normal constituents of milk and cream, especially the minerals, also play a part and the whole process seems to be greatly accelerated by an abundant air supply. Electrolytic action may result when two or more metals having different electrical potentials are in contact with milk or cream which acts as an electrolyte.

Supplee and Bellis [37] noted that the normal copper content of milk was measurably increased by storing or heating in the presence of metallic copper. Slight increases in the copper content also resulted from the passage of milk through the sanitary type of copper pipes from which the tin had been worn off. Rice and Miscall,[38] using for the most part electrolytic determinations, found that the presence of air and oxygen enormously increased the copper dissolved by milk. Corroded copper with an oxide surface yielded much more metal to the milk than smooth, bright copper. Carbon dioxide did not influence the dissolving power of milk. The copper seemed to be associated with the water because, on separating milk containing copper, the copper distributed itself between the cream and skim milk approximately in proportion to the water.

Metals may have an effect on dairy products in a number of ways. Various investigators have pointed out that some of the metallic salts have an intense metallic taste which may directly influence the flavor of materials containing them. It seems probable, however, that as far as butter is concerned the most serious effect of metals results from their action in accelerating certain chemical changes. One of the ways in which this may occur is through the metal acting as an oxygen carrier in an oxidation process, but there are undoubtedly others. Metals may play a part in the destruction of some of the products giving butter a desirable flavor, as well as in the formation of objectionable materials. Presumably the exact effect of the metal is influenced by a number of factors so that there is considerable variation in the final result. Hunziker[39] also considers that metals may have a selective action on organisms and suggests that this may influence the deterioration of butter by favoring the development of objectionable species.

---

[35] Jr. Dairy Sc. 1. P. 320. 1917.

[36] Jr. Dairy Sc. 2. P. 444. 1919.

[37] Jr. Dairy Sc. 5. P. 455. 1922.

[38] Jr. Dairy Sc. 6. P. 261. 1923.

Micro-organisms are involved in the acquisition of metals by milk and cream through the effect of the products of growth, especially acids, on the formation of salts and the rate of electrolytic action. While the processes themselves may be very definitely chemical, the influence of micro-organisms in establishing conditions favorable for them must be recognized.

The appreciation of the importance of metals in causing objectionable conditions in dairy products has resulted in attempts to develop linings for holding tanks, pasteurizing vats, etc. that are very resistant to the changes influencing the metals usually employed. Some of the progressive commercial organizations handling dairy products have found it advisable to frequently retin cans, vats, etc., in order to reduce the amount of metal dissolved.

Enzymatic action in butter deterioration. Cream to be made into butter contains the enzymes that are normally found in milk and also the extracellular enzymes that may have been produced by micro-organisms growing in it or in the milk from which it came. Certain of these enzymes are capable of bringing about the types of changes that are important in butter deterioration. Their effect during the holding of butter undoubtedly varies greatly and is influenced by a variety of factors. The numbers and types of micro-organisms in the milk or cream would be expected to have a very direct relationship to the enzymes elaborated. Pasteurization may destroy some of the enzymes and decrease the activity of others. Temperature has a pronounced influence on enzymatic action generally, so that enzymes might be of importance at one temperature

[39] Chicago Dairy Produce. 32. P. 6. Dec. 15, 1925.

and not at another. Sodium chloride in the concentration in which it is found in butter may also have an effect.

Rogers, Berg, Potteiger and Davis[40] found that in raw cream buttermilk to which chloroform had been added the activity of galactase was almost entirely inhibited by the presence of 18 per cent sodium chloride and a temperature of —17.8° C. (0° F.); when some of the same material was allowed to stand for a considerable period at room temperature the galactase apparently became more active because the casein clotted and the mixture assumed an appearance suggesting that digestion had occurred. A trial with a large quantity of a bacterial enzyme preparation secured from a proteolytic, acid forming organism indicated that milk proteins were hydrolyzed at —6.7° C. (20° F.) in the presence of a high sodium chloride concentration. Pancreatin and pepsin also gave proteolysis under these conditions. Sodium chloride may have had an inhibiting effect in the trials carried out. Combs and Eckles[41] concluded from their studies that the abnormal flavors which developed in butter as a result of mold growth on the cream were caused by enzymes secreted by the mold. *O. lactis* and a Penicillium were used alone and in combination. Pasteurization at 62.8° C. (145° F.) for 20 minutes or 73.9° C. (165° F.) for 5 minutes greatly improved the keeping qualities of butter made from cream on which molds had grown but did not entirely check the action of the enzymes which had been produced. Thatcher and Dahlberg[42] concluded that the normal milk enzymes need not be considered as a factor in the deterioration of butter during storage because of the small amounts in which they are present and the unfavorable influence of storage temperatures on enzyme activity; these investigators found that sodium chloride inhibits galactase to such an extent that this enzyme can not act in normal salted butter.

Relationship of the composition of butter to deterioration. While it is improbable that the variations encountered in the composition of salted butter under commercial conditions have a significant influence on the rate of deterioration, the presence of moisture and curd in butter results in a decrease in the keeping qualities as compared to a more highly purified fat. The ghee made in various countries by heating unsalted butter and then straining, yields a product which keeps very well under severe temperature conditions; the heating very largely evaporates the water and the straining removes a part of the curd which the heat has tended to flocculate. Patil and

---

[40] See ref. 18.

[41] Jr. Dairy Sc. 1. P. 347. 1917.

[42] Jr. Agr. Res. 11. P. 437. 1917.

Hammer[43] found that both ghee and butter fat kept much better than ghee with added water or butter. The addition of water to ghee accelerated deterioration more than the addition of protein.

The water and curd in butter evidently favor the action of bacteria in the deterioration of this product and it seems probable that the water also favors both chemical and enzymatic action. The curd provides a source of objectionable decomposition products.

Effect of carbonating butter on deterioration. In some instances butter has been churned and worked in an atmosphere high in carbon dioxide with the object of destroying objectionable bacteria and improving the quality of the product, both when fresh and after storage. Hunziker[44] concluded that carbonating cream can not be relied on to make such cream or the butter churned from it safe for human consumption through the destruction of injurious bacteria or to destroy organisms that are harmful to the flavor and keeping quality of the butter; he also reported that carbonating does not improve the flavor or market value of butter and that carbonated butter keeps no better than uncarbonated but develops the usual bacterial defects if made from unpasteurized cream.

Prucha, Brannon and Ruehe[45] found in their experiments that the carbonation of sweet cream tended to suppress certain types of bacteria but did not hinder others. The treatment caused a delay in the souring of the cream, the length of which depended on the temperature of holding and probably also on the amount and kind of bacterial contamination; at room temperature the delay was a matter of about two hours while at 1.7° C. (35° F.) several days were involved. When the carbon dioxide was applied at churning time by charging the cream in the churn and replacing the air above the cream with the gas, no significant benefit was noted. The gas caused the fresh butter to taste sour and this flavor tended to cover up the flavor of the neutralizer used. Experiments under both laboratory and commercial conditions showed that the carbon dioxide did not measurably affect the germ life in the butter, improve the flavor or cause it to keep better. The storage of butter in the gas in an air tight container completely inhibited molds in the butter and prolonged the keeping quality at low temperatures but undesirable flavors eventually developed.

The carbonation experiments of Sherwood and Martin[46] indicated

[43] Jr. Dairy Sc. 11. P. 143. 1928.

[44] Jr. Dairy Sc. 7. P. 484. 1924.

[45] Jr. Dairy Sc. 8. P. 318. 1925.

[46] Ia. Agr. Expt. Sta. Res. Bul. 95. 1926.

that carbon dioxide did not improve the quality of butter but showed a tendency to lower the score. The gas had no effect on the bacteria in the butter. The rate of oxidation was as great in the carbonated as in the uncarbonated product.

Valley and Rettger[47] found that the treatment of raw milk with carbon dioxide at atmospheric pressure resulted in little or no inhibition or destruction of bacteria. In pure culture studies with various organisms there was a parallelism between bacteriostatic or bactericidal action and H ion concentration and it appeared that any harmful effect on bacteria from carbon dioxide was due to increased H ion concentration. These investigators concluded that carbon dioxide is necessary for the growth and development of the bacterial cell.

GENERAL TYPES OF CHANGES THAT OCCUR DURING BUTTER DETERIORATION

Although off flavors in butter may result from changes so slight that their detection chemically is impossible with the present methods, certain types of transformations are to be expected from the general nature of the constituents and these suggest some of the possibilities in butter deterioration. The chemical evidence secured in the case of extensive deterioration in butter has confirmed the expected changes in this product.

Protein. The protein of butter may be broken down to compounds of varying degrees of complexity. When extensive enough this decomposition may be detected by determinations of the water soluble nitrogenous compounds, or definite cleavage products, such as amino acids or ammonia, may be determined. The objectionable odors of certain compounds commonly formed in protein decomposition suggest that their elaboration in butter would be responsible for undesirable conditions.

Lactose. Lactose is very susceptible to bacterial action, acid being the important product formed. The conditions in butter are unsuited to the development of *S. lactis* but, since there are other organisms that can attack lactose, the transformation of this constituent is to be expected in certain instances. Pronounced changes in the lactose content may be detected by actual determinations. An increase in the acidity of butter may result from the decomposition of constituents other than the lactose and so can not be interpreted as due to a

[47] Jr. Bact. 14, P. 101. 1927.

transformation of the sugar. The established relationship between acid in cream and deterioration in the butter churned from it indicates that the formation of acid from the lactose in butter would be very undesirable because of its indirect as well as its direct effect.

Fat. The changes that would be expected in the fat of butter are of two types, (1) hydrolysis and (2) oxidation. Hydrolysis results in the formation of free fatty acids and glycerol but with either of these products there may be further changes of various types that result in the development of materials important in causing off flavors and aromas. Attempts are sometimes made to detect hydrolysis by determinations of the acid number but the possibility of acid being produced from other constituents must always be taken into account. In some instances a definite odor suggestive of butyric acid is interpreted as indicating hydrolysis. The oxidation of fat may result in tallowiness, presumably through the effect on the oleic acid radical; this effect may involve changes other than the actual oxidation. Holm and Greenbank[48] found that with pure butter fat the presence of water retarded or prevented the formation of those compounds that produce tallowiness and, although the oxidations in a moist and a dry fat may proceed at the same rate, the dry sample will show tallowiness before the moist. This suggests that variations occur in the direction in which the oxidation proceeds. The iodine number may be used to detect oxidation but this apparently is not influenced by slight changes. Holm, Greenbank and Deysher[49] have secured evidence of loosely bound oxygen compounds in butter oil which they consider are responsible for oxidation in vacuo.

SPECIFIC FLAVOR DEFECTS OF BUTTER

The specific flavor defects of butter may be divided into three groups, (1) those primarily due to unsatisfactory methods in the plant, (2) those primarily due to off flavors in the cream and (3) those that develop primarily during the holding of the butter. This division is not clear cut and a certain flavor sometimes appears under conditions which would class it with a group other than the one it ordinarily belongs with; for example, a fishy flavor commonly develops when butter is held but occasionally is present in freshly made butter. The grouping is useful, however, in that it emphasizes the important causes of the off flavors of butter.

[48] Proc. World's Dairy Cong. 2. P. 1253. 1923.

[49] Ind. and Eng. Chem. 19. P. 156. 1927.

While the undesirable flavors found in many lots of butter are definite and easily recognizable, others are encountered that are indefinite and appear to be unrelated to the usual types of off flavors. In certain instances the latter are combinations of flavors and, accordingly, are difficult of classification.

Flavor Defects Primarily Due to Unsatisfactory Methods in the Plant

Cooked flavor. Fresh butter made from pasteurized cream commonly has a slight cooked flavor but this largely disappears within a few days. In some instances, however, the cooked flavor is pronounced and persists during the holding period so that it is definitely objectionable.

An excessive pasteurization exposure for all or a part of the cream is the primary cause of the cooked flavor. With an excessively high temperature at the heating surface there is a tendency for the cream in direct contact with it to be over heated; when cream adheres to the heating surface and is subjected to high temperatures for excessive periods the cooked flavor is especially pronounced. Butter cultures may show a cooked flavor but are not ordinarily responsible for the condition in the butter in which they are used.

Neutralizer flavor. Butter made from neutralized cream often shows a more or less definite neutralizer flavor. The exact character of this is influenced by the neutralizer used; with lime it may be suggestive of this material while with more active neutralizing agents it may be sharp and, occasionally, it resembles soap.

The extent of the neutralizer flavor is largely determined by the method of neutralizing the cream. If the neutralizer is not properly and quickly distributed, if the neutralization is carried too far or if the cream is heated to a comparatively high temperature before the neutralizer has reacted with the acid present, an undesirable flavor effect is to be expected.

Flat flavor. Butter made with a culture so that it should show a full flavor and aroma sometimes lacks these and is referred to as flat. In general, this is the result of a culture of a poor quality; the lactic acid organisms produce the normal amount of acid but the associated types, which are largely responsible for the flavor and aroma, fail to develop, due to various causes. An under ripe culture always lacks the desirable flavor and aroma and in some instances a flat flavor in butter appears to be directly related to insufficient ripening of the culture used; however, certain cultures characteristically lack flavor and aroma, even with the best of care. A flat flavor is especially noticeable in unsalted butter where the influence of the salt in bringing out the flavor is absent.

The lack of flavor in butter can best be overcome by the making of better culture, either through an improvement in the conditions of handling or by a more careful selection of the original inoculating material.

High acid flavor. A flavor suggestive of considerable acid is sometimes noted in butter and is referred to as a high acid flavor. It is ordinarily the result of an excessive acidity in the cream at the time of churning and this may be due to the condition of either the cream or the butter culture. When a culture simply forms acid, instead of giving the typical flavor and aroma development, there is a tendency for the butter in which it is used to show this condition.

Sour flavor. In some instances the presence of large amounts of buttermilk in butter results in a flavor that is often referred to as sour. This flavor is most frequently noted in butter churned from cream having a high acidity.

Flavor Defects Primarily Due to Off Flavors in the Cream

Feed flavors. Feed flavors in butter are directly related to similar flavors in the cream or, occasionally, in the butter culture. The most serious of these are the flavors due to weeds of various types but many of the very useful feeds, especially certain legumes, also affect the flavor of butter.

Cowy flavor. A cowy flavor in butter may result from this condition in the cream. It should be recognized that a cowy flavor in milk or cream may be due to the growth of certain bacteria as well as to the absorption of odors in the stable.

Yeasty flavor. A yeasty flavor in butter is primarily due to the development of the lactose fermenting yeasts, *T. cremoris* and *T. sphærica,* in the cream before it reaches the plant, although careless holding of the cream in the plant may also result in the yeasty condition or intensify it if already present. These organisms require comparatively high temperatures for their rapid multiplication so that yeasty cream is more or less definitely limited to the summer months. It may, however, be present during cold weather in cream that is held warm. The conditions making possible the growth of *T. cremoris* and *T. sphærica* also result in the rapid growth of the lactic acid bacteria so that the typical yeasty flavor is noted only in sour cream.

The control of the yeasty flavor in butter involves the prevention of the growth of the yeasts in the cream; this can best be accomplished through the use of lower holding temperatures or more frequent deliveries.

Malty or burnt flavor. A malty or burnt flavor in butter is ordinarily the result of the growth of *S. lactis var. maltigenes* in the cream. This organism has also been found at the Iowa Agricultural Experiment Station in off flavored butter cultures sent in from creameries. Cream may show a malty flavor before there has been a significant increase in acidity and the condition has been noted at plants receiving a very high grade of raw material. In some instances the cream from a certain farm quite regularly shows this flavor, due presumably to a rather constant infection with the responsible organism. The flavor occurs most often during the warmer months but is by no means limited to that season.

The malty or burnt flavor can be prevented by controlling the growth of *S. lactis var. maltigenes* through more careful methods of producing and holding the cream.

Moldy or musty flavor. Butter commonly shows a moldy or musty flavor when molds have grown on it but this may also occur without such development, due to the absorption of the flavor at some stage in the handling of the cream or butter. A moldy flavor may be present in cream as a result of it being held in a cellar having a musty odor or molds may grow on cream subjected to favorable growth temperatures for extended periods. The plant treatment of such cream may decrease the intensity of the condition but can not remove it completely. A musty odor in such equipment as churns may also be absorbed by the butter. In some instances molds may be actively growing in the butter culture but such growth is quickly detected by the experienced culture judge.

When the moldy flavor comes from the cream it can be controlled only by an improvement in the methods of handling this material. It is impossible to keep mold spores out of cream with the ordinary methods of production so low holding temperatures must be used to prevent growth and should involve an atmosphere free from a musty odor. Proper control of the growth of molds in the plant equipment will eliminate the absorption of a moldy flavor during the handling of the cream and butter.

Old cream or stale flavor. Certain lots of butter show a flavor that is difficult to describe but which suggests that the cream used was of a poor quality. This is frequently referred to as an old cream or stale flavor. The flavor is very definitely due to the growth of micro-organisms of various types in the cream; the products of this growth are, in part, destroyed by neutralization and pasteurization but can not be entirely overcome and influence the flavor of the butter.

The prevention of the old cream flavor involves better methods of producing and holding the raw material.

Metallic flavor. A very characteristic condition that is referred to as metallic is sometimes present in butter and may be detected by either the flavor or aroma. It is also very common in buttermilk. The flavor suggests the astringent effect given by certain metals when they are taken into the mouth.

The metallic flavor in butter is due primarily to the same condition in the cream at the time of churning. The flavor may be present in cream as it comes from the producing farms, especially in the spring and fall, or it may be the result of the growth of organisms during holding in the plant. A metallic flavor in cream often disappears when the cream is held under conditions very favorable for the growth of micro-organisms, due presumably to it being covered up by some more objectionable flavor. Because of its relationship to the condition of the cream, a metallic flavor in butter is usually present as soon as the butter is made. In buttermilk, however, the flavor readily develops if the product is held at certain temperatures.

Guthrie[50] studied the development of a metallic flavor in dairy products and concluded that the direct absorption of metals or the action of bacteria may be responsible. He reported that the organism involved is a strain of the *S. lactis* group. High acidity was a necessary condition for the development of a metallic flavor. This investigator pointed out the interesting fact that, except in the case of buttermilk, a high fat content of the medium is essential for the development of a metallic flavor. It was found that the condition may develop in cream of either a good or poor flavor.

Baker and Hammer[51] investigated the production of a metallic flavor in buttermilk and found that it may occur when the buttermilk is held in a glass container so that the absorption of metal is improbable. A metallic flavor was produced in sterile buttermilk by the inoculation of a butter culture or a mixture of *S. lactis* and either *S. citrovorus* or *S. paracitrovorus.* Uninoculated buttermilk or sterile buttermilk to which butter culture had been added became metallic

[50] N. Y. (Cornell) Agr. Expt. Sta. Bul. 373. 1916.

[51] Proc. Ia. Acad. Sc. 32. P. 55. 1925.

more frequently at 12° C. (53.6° F.) than at room temperature. These results indicate that the normal butter culture organisms are capable of causing a metallic flavor when their development is excessive.

It seems probable that the flavor referred to as metallic may be due to more than one cause. The absorption of traces of metals from cans and equipment of various sorts, especially when such easily attacked metals as iron and copper are exposed, is presumably a factor in certain instances. This absorption is favored by the presence of acid and probably also by exposure to air. Excessive bacterial action of the normal culture organisms may also cause a metallic flavor. Because of the unfavorable conditions in butter for the development of most organisms this growth ordinarily takes place in the cream. In many instances a metallic flavor has been noted in butter churned from cream ripened to a high acidity and this has sometimes occurred when both the cream and the butter culture were of a satisfactory quality.

The metallic flavor in butter is usually soon replaced by some other flavor and is uncommon in storage butter. Fishiness often follows it and, since excessive acid in the cream is related to the fishiness, the importance of acid in causing the metallic flavor is suggested.

Flavors distinctly foreign to butter. Butter occasionally shows a flavor distinctly foreign to it, such as kerosene, gasoline, fly spray or disinfectant flavor. This is usually due to the absorption of odors by the cream but there may be an actual addition of some foreign material through carelessness. Absorption by the butter may also occur but there is less likelihood of this than of absorption by the cream on the producing farms.

Flavor Defects That Develop Primarily During the Holding of the Butter

Storage flavor. Butter which has been held in storage for a considerable period commonly shows a flavor which the experienced butter judge recognizes as a storage flavor. The intensity varies greatly and, with properly made butter, may be very slight. This condition is probably due to slow chemical changes; although such changes can be reduced to a minimum by a high quality of raw material and by proper manufacturing methods, it appears that they can not be entirely prevented and some deterioration must be expected in butter when it is stored for an extended period.

Tallowy flavor. Butter sometimes develops a flavor and aroma suggestive of tallow and, when these are pronounced, the product is often bleached. In general this condition appears in butter held in retail establishments or homes and not in butter during storage.

When butter or butter fat is exposed to the sunlight the surface develops a tallowy flavor and aroma and bleaches in a short time. The condition also appears in diffuse light but requires a longer exposure. These results suggest that tallowiness is due to the action of air and that sunlight increases the rate; temperature would also be expected to have an influence. The development of tallowiness in sterile butter fat indicates that the action of micro-organisms is not directly involved.

According to the findings of various investigators, the changes occurring during the development of tallowiness result in a decrease in the iodine number. This has sometimes been assumed to indicate that tallowiness is due to the oxidation of the oleic acid. Hunziker and Hosman[52] have pointed out, however, that butter does not show a tallowy flavor when oleic acid, which has been exposed to the air until there has been a reduction in the iodine number, is added to it but that butter containing free oleic acid rapidly becomes tallowy when exposed. These investigators accordingly consider that tallowiness is not the direct result of oxidized oleic acid but that it is due to products formed in the butter during the absorption of oxygen by the oleic acid; since glycollic acid can be derived from the oxidation of glycerol, esters of this compound are a possible cause of tallowiness. Trials showed that, although the addition of glycollic acid did not give butter a tallowy flavor, glycollic acid esters of the fatty acids of butter produced a tallowy flavor when warmed with butter fat, especially the glycollic acid ester of oleic acid. The tallowiness was produced in the absence of air and comparison samples, in which oleic acid alone was added, showed no tallowiness. The small amount of glycollic acid ester required to produce tallowiness is shown by the fact that 0.25 per cent of this reagent added to butter fat produced a distinctly tallowy flavor and aroma. The relationship of glycollic acid to tallowiness is suggested by the presence of glycollic acid in the fat of sheep's wool which has a tallowy odor.

Various factors which may influence oxidation would be expected to have an effect on the development of tallowiness. Hunziker and Hosman have shown that metals and metallic salts are important in this connection through their action as oxygen carriers and that over neutralization may make certain materials susceptible to oxidation.

---

[52] See ref. 35.

Rancid flavor. Butter sometimes develops a flavor and aroma suggestive of butyric acid and is then described as rancid. The condition seems to involve primarily the fat. The rancidity in different lots of butter shows variations and, in some instances, the condition is by no means characteristic, so that there may be a difference of opinion among butter judges as to the designation of the defect. Guthrie[53] has suggested that rancidity, as defined by butter dealers and expert judges, is rarely found and that the average person thinks of a strong flavor in butter as rancidity. Materials other than butter may develop rancidity and this can occur with practically pure fats. Micro-organisms constitute an important cause of the hydrolysis leading to rancidity but lipase and probably chemical action may also play a part.

The studies of Orla-Jensen[54] have shown that micro-organisms can produce rancidity in butter. This investigator found that a number of species are capable of doing this, including *O. lactis, Cladosporium butyri, Ps. fluorescens* and *S. marcescens,* and that all of these hydrolyze fat. Rancidity may develop under conditions which apparently exclude the growth of micro-organisms so that other causes are indicated. The investigations of Rice,[55] which show the importance of lipase in the production of rancidity in sweetened condensed milk, suggest that this enzyme may be of significance in the deterioration of butter at favorable temperatures. In trials carried out by Guthrie,[56] however, enzymes did not produce rancidity.

It seems to be impossible to measure chemically the development of the products responsible for rancidity. While rancid butter may show considerable acidity, as determined by titration, this is not always the case and, moreover, there may be considerable acidity in butter without a suggestion of rancidity. Any relationship that might be expected between acidity and rancidity in butter is greatly complicated by the fact that there are sources of acid in butter other than the fat. It appears also that the products responsible for rancidity need to be present in only very small amounts. Various investigators have considered that the hydrolysis of fat is only a step in the development of a typical rancid condition and that other changes must follow.

---

[53] Jr. Dairy Sc. 1. P. 218. 1917.

[54] Centbl. f. Bakt. 2 Abt. 8. Pp. 11, 74, 107, 140, 171, 211, 309, 342, 367, 406. 1902.

[55] Jr. Dairy Sc. 9. P. 293. 1926.

[56] See ref. 53.

Rancidity may develop rapidly at comparatively high temperatures and it seems probable that micro-organisms are then the important causative factor. The condition may also appear in storage butter where the growth of micro-organisms is very improbable.

Hunziker [57] has pointed out that in studies on the pasteurization of cream carried out by Hunziker, Mills and Spitzer, the butter made from pasteurized cream did not become rancid during storage, that made from raw ungraded cream almost invariably showed this condition while that made from raw selected cream occasionally showed it. These results illustrate the importance of both the quality of the raw material and the treatment given it in the prevention of rancidity.

Fishiness. The development of a fishy flavor and aroma in butter is one of the very serious types of deterioration. It is primarily a defect of butter that has been stored but may also appear in fresh butter. Some of the heavy financial losses in storage butter have been due to the development of pronounced fishiness in butter that went into storage in an excellent condition. Fishiness also occurs in dairy products other than butter.

The early investigations of fishiness led to various conclusions. O'Callaghan [58] cited experiments which suggested that *O. lactis* is the cause of the condition. Later [59] he reported that when this organism was grown in conjunction with *S. lactis* fishiness was produced on every occasion; he also noted that many consignments of unsalted Australian butter had turned out fishy in London and that fishiness is sometimes detected in unsalted butter before it leaves Australia. The idea that salt may cause fishiness has been considered by Piffard,[60] who pointed out the ability of salt to absorb the odor of material stored near it. This author also stated that a fishy flavor in milk is undoubtedly sometimes the result of the flavor in water to which the cows have access and which is due to the development of diatoms and algæ.

Rogers [61] concluded from his study of fishy butter that the flavor is evidently not produced by the action of any one special factor

[57] Hunziker, O. F. The Butter Industry. 2nd ed. P. 517. 1927.

[58] Agr. Gaz. N. S. Wales. 12. P. 341. 1901.

[59] Agr. Gaz. N. S. Wales. 18. P. 223. 1907.

[60] N. Y. Prod. Rev. 13. P. 20. 1901.

[61] See ref. 27.

and that the results indicate its immediate cause is a substance produced through the oxidation of one of the combinations of the acid developed in the ripening of the cream. He noted that acid is essential and that butter made from pasteurized sweet cream with a butter culture, but without ripening, seldom if ever becomes fishy; over working the butter made from sour cream produced a fishy flavor with reasonable certainty. This investigator was unable to produce fishy butter by the inoculation of *O. lactis* into the cream and, in samples of fishy butter examined, it was never found in more than very small numbers.

Reakes, Cuddie and Reid[62] were unable to find significant differences between the bacteria of fishy and of high grade butter. Moreover, plugs of fishy butter put into high grade butter failed to cause fishiness and plugs of high grade butter placed in tubs of fishy butter retained their desirable flavor. These investigators concur with Rogers in the idea that a chemical change results in a splitting up of some of the butter constituents into compounds having a fishy flavor and aroma.

Rogers[63] has pointed out that fishiness is usually preceded by an oily or metallic flavor and that it is especially common following conditions that produce more than the usual amount of sour cream. He considered the evidence against the trouble being bacterial in nature and definitely ascribed it to a chemical change favored by high acid and the presence of air and such materials as salts of iron and copper that may act as catalytic agents.

The fishy odor of trimethylamine naturally suggested this compound as a cause of fishiness in butter. Although Rogers[64] was unable to produce fishiness by working large quantities of it into butter, Supplee[65] secured a fishy flavor when the trimethylamine was worked in under proper conditions, especially when trimethylamine butyrate was used. Supplee noted that normal butter contains lecithin, which can yield trimethylamine on decomposition; trimethylamine was found in some samples of fishy butter but not in others. Sommer and Smit[66] reported that the addition of trimethylamine lactate to butter caused a distinct fishy flavor and that trimethylamine could be isolated from the samples of fishy butter examined; butter made from cream to which lecithin had been added was found to become fishy more readily than butter made from untreated cream of the same lot. These investigators noted that organisms which produced trimethylamine from lecithin under favorable conditions failed entirely to grow in the presence of salt and acid in such concentrations as would be found in the brine of butter.

---

[62] Jr. New Zealand Dept. Agr. 4. P. 1. 1912.

[63] Proc. 13th An. Meet. Wis. Buttermakers' Assn. P. 70. 1914.

[64] See ref. 27.

[65] N. Y. (Cornell) Agr. Expt. Sta. Mem. 29. 1919.

[66] Wis. Agr. Expt. Sta. Res. Bul. 57. 1923.

With chemical decomposition of lecithin the probable cause of fishiness under storage conditions, the factors influencing this process become of importance. Acid favors the hydrolysis of lecithin and may increase the content of metals which can act as catalysts. A salt solution is a good solvent for lecithin and thus may get the lecithin in such a condition that chemical decomposition can go on more rapidly; this may explain why fishiness has been so much more frequently noted in salted butter than in unsalted. Over working favors the solution of the lecithin and may distribute the incorporated air more uniformly. Sommer and Smit[67] have pointed out that pasteurization tends to eliminate fishiness because it causes some hydrolysis of the lecithin, the products of which are lost in the buttermilk and because, during the heating, the lecithin absorbs oxygen and becomes less soluble.

Cusick[68] reported that a fishy flavor was produced in butter by inoculating the cream with *Bac. ichthyosmius,* an organism that was originally secured from fishy evaporated milk. The age and acid content of the cream and the period of incubation before churning seemed to influence the production of a fishy flavor in the butter.

Surface taint. The term ''surface taint'' is used to refer to the development in butter of an objectionable flavor and aroma suggestive of putrefaction. The defect begins at the surface and, at comparatively high temperatures, may develop rapidly. It has been noted especially in Canada and, according to Hood and White,[69] made its appearance there in 1919 in first grade, pasteurized cream butter sent to the Vancouver market; in the western provinces it has become an important problem while in Ontario and Quebec it has been rather rare.

Eckles[70] reported an outbreak of putrid butter that occurred in 1900 and lasted about two weeks. The defect was so pronounced the local sale of butter was stopped and what had been sold was returned.

---

[67] See ref. 66.

[68] Jr. Dairy Sc. 3. P. 194. 1920.

[69] Dom. Can. Dept. Agr. Pamphlet 91. n. s. 1928.

[70] Ia. Agr. Expt. Sta. Bul. 59. 1901.

The plant was kept in a good condition but the milk received was of an unsatisfactory quality. Changes involving the rejection of poor quality milk, better care of utensils and the use of a butter culture were instituted at the plant and about the same time a long, dry period was broken by heavy rains. The butter improved at once and no further difficulty was experienced. The putrid butter contained an abnormally high number of gelatin liquefiers which included some forms that had a very injurious effect on butter. Eckles concluded the putrid condition was brought about by contamination of the milk.

The data available suggest that surface taint in butter is due to the development of micro-organisms. Hood and White[71] noted that butter with this defect showed abnormally high yeast and bacterial counts, and that all the samples contained large numbers of bacteria capable of decomposing curd. It was also found that mold, yeast and bacterial counts on butter from plants having difficulty with surface taint were usually high, although with some factories variations in the results suggested inconsistent sanitary control. Many samples of raw cream butter examined contained fewer bacteria than surface taint butter made from pasteurized cream. Nothing abnormal was noted in the acid or curd content of butter showing the defect. These investigators produced surface taint in experimental butters made from pasteurized, neutralized cream by inoculating, into the cream, certain bacteria isolated from water; without inoculation the defect did not develop.

Careful plant methods so that the butter will contain but few molds, yeasts and foreign bacteria are the suggested methods of controlling surface taint. Mold and yeast counts afford a convenient method of determining the care with which the plant operations are performed.

COLOR CHANGES IN BUTTER DUE TO MICRO-ORGANISMS

Color changes may occur in butter as a result of the growth of micro-organisms. Various types of molds are especially important in this connection while, occasionally, the pink yeasts have been involved. These color changes never take place rapidly but occur more readily in unsalted butter than in salted because of the absence of the restraining action of the salt.

Moldy butter. The development of molds occasionally occurs with both print and tub butter. Growth is largely confined to portions at or very near the surface, due to the oxygen requirements of the organisms, although rarely an air space may permit of some growth down in the butter mass. Mold in butter is the cause of considerable loss because the moldy portion must be removed, thus involving a decrease in weight and additional labor, and also because of the influence of the mold growth on the flavor of the normal appearing

---

[71] See ref. 69.

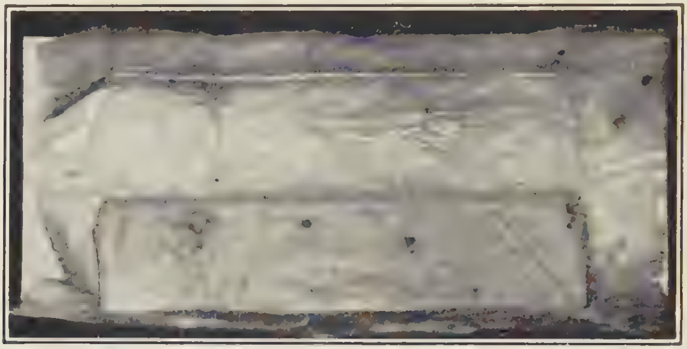


Fig. 35.—Slight mold development on quarter pound print of butter.

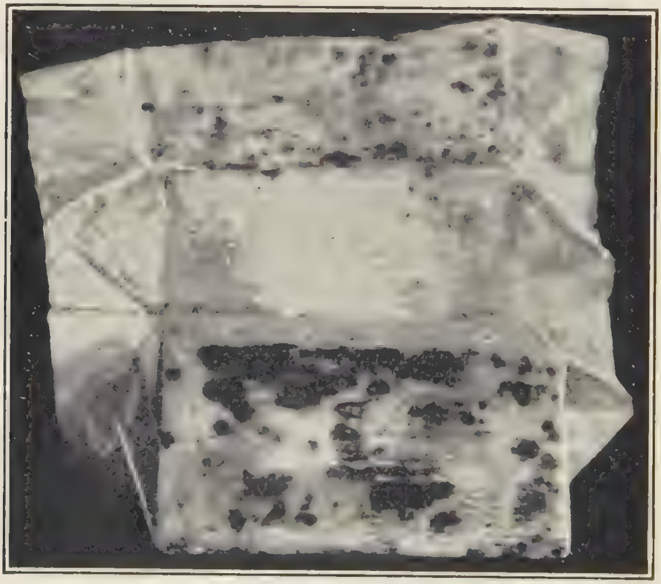


Fig. 36.—Excessive mold development on pound print of butter.

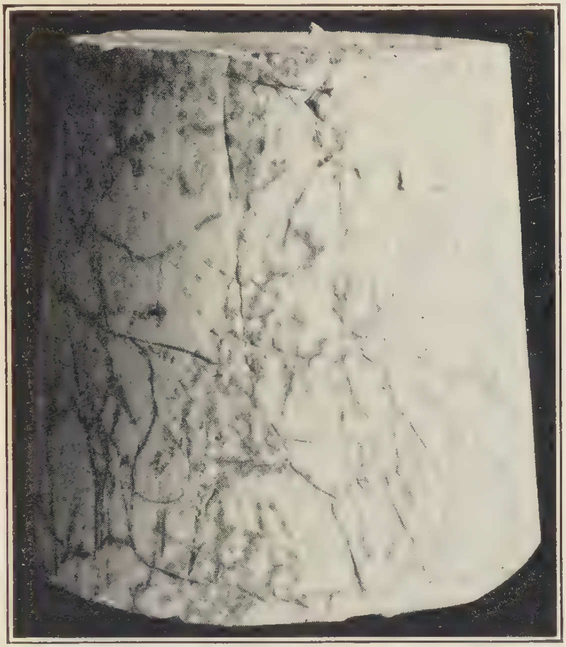


FIG. 37.—Mold development on tub butter. Courtesy H. Macy.

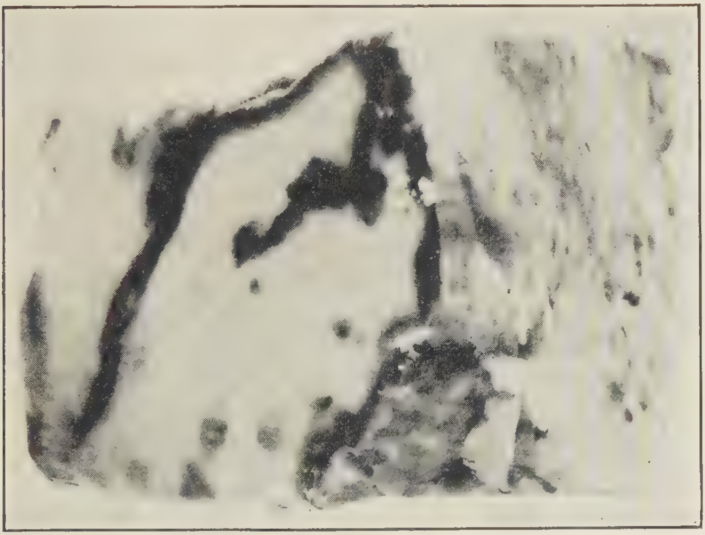


FIG. 38.—Cut surface of butter showing unusual mold growth through the butter mass. Courtesy H. Macy.

portion of the package. This defect is most common during the warm months when the temperature is especially favorable for growth. In general, moldy butter is an indication of unsatisfactory methods of manufacturing and packing. However, various factors, such as high holding temperatures, may be the immediate cause of the condition; in the past, unsatisfactory car refrigeration has repeatedly resulted in moldy butter reaching the markets in large quantities. There appears to be a great variation in the prevalence of mold in butter from year to year as well as from season to season.

Types of molding. The moldy areas that develop on butter vary widely in appearance, due to differences in the species involved and also in the extent of growth. In some instances two or even more molds may be growing in combination and this associative action has a pronounced influence on the character of the growth.

Thom and Shaw[72] have recognized three main types of mold growth as follows:

1. Smudged or Alternaria type. In this type of molding there are dark, smoky or rarely greenish-colored areas suggesting soot or dirty finger marks. Sometimes hyphæ were observed four or five millimeters below the surface. Spores were rarely found but colonies transferred to culture media grew freely and fruited normally. The dark brown or black hyphæ were the most common and proved to be species of Alternaria; from the greenish-colored areas species of Cladosporium developed.
2. Green type. Green molds sometimes develop on the surface of butter and follow seams or cracks down into the butter mass. The mold may also be present on the liners, circles or the tubs. The cultures secured from this type of molding proved to be species of Penicillium.
3. Oidium type. Various shades of orange and yellow discoloration showing little or no surface growth also develop on butter. Cultures and microscopic examinations showed that this condition was due to *O. lactis*. The organism grew to a depth of several millimeters within the mass of butter as a complex mycelium with hyphæ that varied in diameter according to the size of the spaces between the masses of fat. Some spores were formed and at times there was surface fruiting.

Sources from which molds get into butter. Molds may get into butter from various sources. Raw cream, either sweet or sour, practically always contains these organisms, due to contamination from the air, utensils, etc. In the butter plants the cream may have molds added to it from improperly cared for equipment such as churns, vats, pumps and piping; churns are a common and sometimes a very serious source of molds because of the difficulty of entirely preventing the growth of micro-organisms in them. Occasionally, molds are added to cream through the use of butter cultures containing these organisms. The cream is not the only source from which molds get into butter and certain lots are contaminated from the packing materials, such as tubs, liners, circles and wrappers, and from the equipment used in packing and cutting butter. The hands and clothing of persons working with the butter must be considered as possible sources of molds and this is especially true in the smaller plants where the work is not specialized and an employee may handle raw cream and then, a moment later, pack butter. Such materials as salt and wash water that come in intimate contact with all portions of the butter may also be involved.

---

[72] Jr. Agr. Res. 3. P. 301. 1915.

Macy and Combs[73] carried out a series of field studies on the sources of molds in Minnesota creameries and found that, in all cases, the raw cream was heavily contaminated, even when most of it was sweet and fresh. About 90 per cent of the dry parchment and cloth circles were contaminated. In nearly 75 per cent of the creameries the piping and pumps were adding molds to the cream while in over 65 per cent the churns were infected. Water was a source of molds in 44 per cent of the plants, butter culture in 40 per cent and salt in 33 per cent.

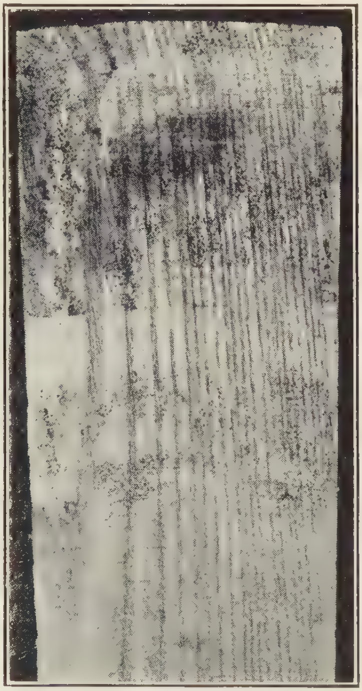


FIG. 39.—Mold development on tub stave.

Hood and White[74] found that the interior of moldy butter contained large numbers of yeasts and molds which indicates that the butter must have been heavily contaminated. In the examination of a series of samples of butter, some of which were made from raw cream and some from pasteurized cream, these investigators found no appreciable difference in the content of yeasts and molds, due undoubtedly to the extensive recontamination of the pasteurized cream.

---

[73] Minn. Agr. Expt. Sta. Bul. 235. 1927.

[74] Dom. Can. Dept. Agr. Bul. 48. n. s. 1925.

Preventing the contamination of butter with molds. The production of cream free from molds is impossible under practical conditions and proper pasteurization is the advisable method of controlling the contamination of butter from this source. The studies of Thom and Ayers[75] have shown the general resistance of mold spores to heat. When heated for 30 minutes in milk a number of species were killed at 54.4° C. (130° F.) while a few were destroyed at a lower temperature. Several species survived at 60° C. (140° F.),

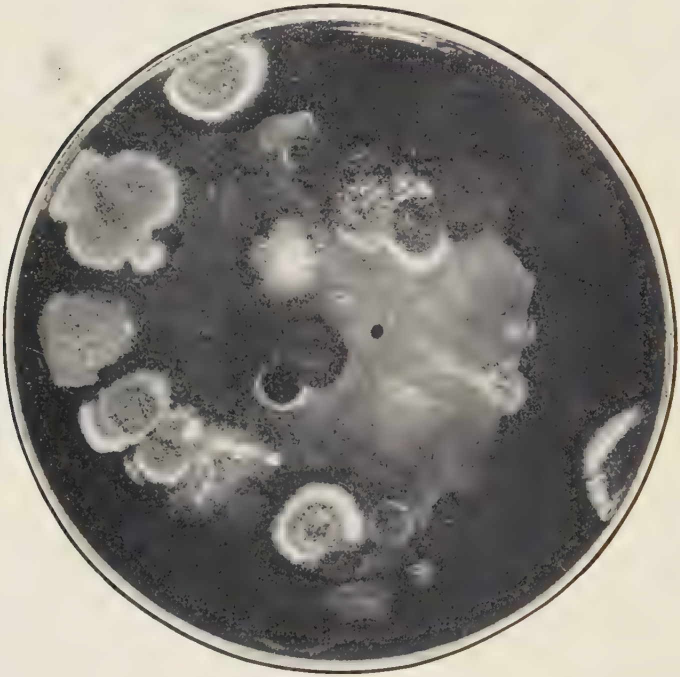


FIG. 40.—Mold development following the covering of contaminated parchment with agar.

---

[75] Jr. Agr. Res. 6. P. 153. 1916.

but 62.8° C. (145° F.) was found to destroy mold spores completely enough to render them a negligible factor in the future changes in milk. With a heating period of 30 seconds, which represents the conditions in flash pasteurization, 62.8° C. killed very few species, 68.3° C. (155° F.) killed most species, while 73.9° to 79.4° C. (165° to 175° F.) killed practically all, although some species occasionally survived. Dry heat proved to be much less effective; with a 30 minute period, 82.2° C. (180° F.) killed very few species and some even survived 121.1° C. (250° F.). From these results it is evident that the molds in cream can quite readily be destroyed by proper pasteurization. The ineffectiveness of the pasteurization in certain creameries from the standpoint of mold destruction is shown by the results of Macy and Combs[76] who found the molds had not been destroyed in 40 per cent of the samples of supposedly pasteurized cream from Minnesota creameries.

Preventing the contamination of cream in the plant involves primarily the proper care of equipment. The churns need special attention because of the difficulty of keeping wood that is regularly moist free from micro-organisms and also because the angles, bolt heads, etc. on the inside tend to collect material that supports growth. As soon as the churning is completed the churn should be rinsed well with clean, hot water and then washed with plenty of hot water and washing powder; during this washing the water should be brought to the boiling point with steam and the churn then revolved for several minutes in high gear. After the wash water is drained the churn should be well rinsed with an abundance of boiling water by revolving it in high gear with the water in it. The churn should then be thoroughly drained and left with the open doors up so that drying can take place. In some instances it may be advisable to rinse the churn shortly before use, first with hot water and then with cold water.

The vats should be thoroughly cleaned and steamed; between periods of use they should be dry so as to prevent the growth of organisms in them. The pumps and piping require thorough washing and sterilization and should be taken down for this. It is advisable to get the parts hot enough by the sterilization so that the moisture which does not drain away will evaporate, thus leaving them in a dry condition. If there is any question about the condition of the piping and pumps they should be thoroughly treated with hot water or steam just before use.

[76] See ref. 73.

With careful handling the butter culture should not contain molds. The presence of these organisms in a mother culture can be detected by allowing it to stand for a number of days at room temperature and observing for mold growth at the surface. Salt should be stored under conditions which control contamination with molds; this requires a clean, dry room so that the spores in the air will be very few in number.

The materials used in packing butter, such as tubs, liners, circles, etc., should be stored under conditions which prevent excessive contamination and then treated before use. The tubs may be soaked and then thoroughly scalded out. The liners and circles can conveniently be heat treated by putting them in boiling water or brine and wrappers can be handled in the same way. A satisfactory method is to have a covered container, such as a stone jar, filled with brine into which these materials can be placed and the temperature then raised with steam. By allowing a long cooling period there is no inconvenience in handling the materials; some small creameries find it advantageous to carry out the heating the day before. The salt is of no value in destroying molds but keeps down the growth of organisms in the water so that the same water can be left in the tank for some time; moreover, certain buttermakers consider that liners handle more easily when they have been in salt solution. Chemicals are sometimes recommended for the treatment of materials used in packing butter but have no advantages over heat.

Growth of molds in butter. The contamination of butter with mold is not necessarily followed by growth and many lots of butter showing excessive mold counts have a normal appearance for extended periods. More or less definite requirements must be met before growth can occur and these are not always provided in butter.

Molds grow on a variety of materials, many of which are comparatively resistant to the action of micro-organisms, so that the nutrients supplied by butter are undoubtedly satisfactory. It seems that the fat is less suitable for growth than the curd and it may be that some of the butter constituents which are present in very small amounts are especially important in this connection. The salt present in butter has an inhibiting effect on mold development as is shown by the fact that mold is much more troublesome in unsalted butter than in salted. The early studies of Gripenberg[77] recognized the importance of salt in controlling mold growth and all subsequent investigations have emphasized it. Thom and Shaw [78] concluded that salt up to from 2.5 to 3 per cent in butter, which is equivalent to a 12 to 15 per cent brine, is sufficient to eliminate mold or reduce it to a negligible amount. Investigations have shown, however, that many lots of butter containing these amounts reach the markets in a moldy condition. Hastings [79] has explained this by pointing out that amounts of salt which effectively prevent the germination of mold spores do not stop the growth of vegetative cells; accordingly, if pieces of mold filaments are present in or on butter, as a result of contamination from some source, these may continue their development in the presence of considerable salt, although the salt undoubtedly decreases the rate of growth. Boekhout and Ott de Vries [80] also noted the restraining action of salt on mold cultures secured from moldy butter.

---

[77] Milch-Ztg. 28. Pp. 626, 644, 662. 1899.

The temperature has a very direct influence on the development of molds. In general, growth is progressively reduced as the temperature is decreased from room temperature, until at 0° C. (32° F.) it is very slow if it occurs at all. Under practical conditions the rapid development of molds takes place principally when the butter is held at temperatures that are comparatively high for storage.

A high humidity favors the growth of molds as is evident from the frequent development of these organisms in damp places, such as cellars. Inoculations made from moldy butter to normal butter in a petri dish often fail to grow under room conditions but show a good development in a moist chamber. The moisture content of the air seems to be more important than that of the material on which the molds are growing and, with a favorable humidity, molds may develop on nutrients that are very low in water. Thom and Shaw [81] found that molds would not grow on butter exposed to humidities of 70 per cent or lower while a species of Penicillium grew on a mixture of butter fat and skim milk powder held at a favorable humidity. The air in butter packages has an opportunity to develop a high humidity because it is more or less confined and can take up water from the butter and moist packing materials, especially when the butter does not properly retain the moisture. The removal of butter to a lower temperature raises the relative humidity of the air spaces.

Molds require free oxygen for their development and, accordingly, mold growth in butter is confined to portions where air is available. Occasionally, growth penetrates several millimeters away from the surface in contact with an oxygen supply. Cracks left by careless packing may be a factor in mold development because the air supply which they provide favors both initial mold growth and the spreading of that which occurs. Butter, especially if it is leaky, tends to collect water next to the tub and when this drains away air spaces of considerable size may result. The paraffining of tubs has its advantage, in part, in preventing the loss of water either through absorption by the wood or actual drainage and thus controls the formation of air spaces.

---

[78] See ref. 72.

[79] Address Wis. Buttermakers' Assn. Dec. 5, 1916.

[80] Centbl. f. Bakt. 2 Abt. 52. P. 39. 1920.

[81] See ref. 72.

Pink yeasts as a cause of color changes in butter. In some few instances pink yeasts have been responsible for a color change in butter. This appears either as a pink tinge over a considerable area or as small sharply defined spots. Investigations have shown that at least some of the pink yeasts develop better in unsalted butter than in salted.

CHAPTER XV

BACTERIOLOGY OF CHEESE

The making of cheese appears to have begun in an attempt to store important milk constituents during a period of abundance for use when the milk supply was limited. Variations in the character of the curd secured and in the curing procedure favored the growth of different groups of organisms and, accordingly, more or less definite types of cheese originated. Although methods of making cheese developed long before the importance of organisms in dairy products was recognized, an understanding of their action in the various cheeses is, at present, necessary because of the market demands for a uniformly high quality and also because cheeses are now made in localities where some of the natural advantages, under which the making began, are lacking.

A great many factors, such as the temperatures used during the manufacture and curing, the per cent water, the per cent salt and the humidity, have a pronounced effect on the organisms developing in cheeses and variations in these factors explain the large number of varieties that are made.

Classification of cheeses. The various cheeses may be divided into two groups, (1) those with which the curdling of the milk is primarily due to the action of rennet and (2) those with which the curdling is primarily due to acid development. The rennet curd cheeses may be classified, on the basis of the moisture content, into two types, (1) the hard cheeses and (2) the soft cheeses; the moisture retained during the manufacturing, in combination with other factors, has a pronounced influence on the growth of micro-organisms and this affects the general character of the ripening. The dividing line between the hard and soft rennet cheeses is not sharply defined and there is a gradation from the typical hard cheeses over to those that are typically soft.

With the hard rennet cheeses the ripening goes on quite uniformly throughout the mass, except as influenced by such factors as drying at the surface, and if desired they can be made in large sizes. The ripened product is not susceptible to the rapid action of objectionable micro-organisms and, as a result, has good keeping qualities. With the soft rennet cheeses micro-organisms prominently connected with the ripening develop at the surface and the products of growth diffuse in through the cheese mass and effect the characteristic changes; the necessary diffusion accordingly limits the size of the cheese. The opportunity for continued growth of micro-organisms in the soft cheeses, even after the desired ripening has occurred, results in the development of objectionable conditions of various types and the keeping quality is rather limited.

The acid curd cheeses require no ripening since the necessary bacterial products are formed during the manufacturing. These cheeses are at their best soon after being made and, in general, any further growth of micro-organisms results in deterioration. The yeasts and molds find conditions very favorable for their development and often cause undesirable changes.

General ripening agents. The general changes that occur in the ripening of cheeses involve the action of micro-organisms and the enzymes normal to milk or added with the coagulating agent. More than one organism is ordinarily concerned with the ripening of a variety and the changes brought about by the various species may be very different. The products resulting from the action of one of the ripening agents sometimes constitute the factor making possible a later change; in this way there may be a regular sequence of changes occurring during the ripening.

Some of the early work on the ripening of cheeses led to erroneous ideas as to the causative agents. Bits of old cheese were inoculated into sterile milk and yielded a coagulation and cheesy odor which led to the assumption that the spore bearing rods appearing in the milk were an important factor in the ripening process. These organisms apparently belonged to the sweet curdling spore formers that so commonly give a cheesy odor in milk; they controlled the fermentation in the inoculated milk because the bacteria that are the important ones in the ripening are not spore formers and do not survive the conditions existing in the old cheese in such a way that they can grow rapidly when introduced into sterile milk. Later investigations quickly showed the presence of large numbers of acid formers during the ripening of various cheeses and this suggested that these forms are important. In general, the organisms that grow well in milk also grow well in cheese and the acid formers are the first group of organisms to develop during the ripening of many varieties.

General distribution of organisms in cheese. When milk coagulates, either with acid or rennet, the contained organisms are held quite firmly so that the growth which subsequently occurs results in the formation of colonies comparable to the colonies in an agar plate. The organisms in a cheese are, accordingly, more or less unevenly distributed and, although a type which was very numerous in the milk may be expected in each tiny bit of cheese, another type may not be present in a comparatively large portion, even after rather extensive multiplication. The growth of an important micro-organism may be definitely limited to a certain portion of the cheese by some such factor as the air supply; this is best illustrated by the cheeses in which the development of molds normally occurs. There is a diffusion of materials to and from the colonies of micro-organisms so that the ripening is not confined to the immediate neighborhood of the masses of growth.

Determination of the number of bacteria in cheese. The number of bacteria per gram of cheese is ordinarily determined by the plate method. The portion for examination is weighed out on a sterile paper and ground in a sterile mortar with some material which helps to disintegrate it, after which the mass is transferred to a water blank. The blank is then thoroughly shaken and the dilutions made the same as with milk. Approximately one gram is a convenient sample but the exact quantity is immaterial, as long as the weight is known so that the final results can be calculated to a gram basis. With the hard cheeses the sample can ordinarily be completely removed from the paper while, with certain soft cheeses, it may be necessary to grind up the paper with the cheese; if the latter procedure is used, the paper is protected from contamination by having a larger sterile paper on the balance pan.

When possible, the sample of cheese for analysis is taken from a plug drawn with a sterile trier after carefully scraping away the surface portion of the cheese, from the area to be bored, with a sterile knife or spatula. It is preferable to make up the sample from various places in the plug so that the different parts of the cheese will be represented and, in some instances, cheese is taken from several plugs. The pieces of cheese used must be cut from the plug with a sterile knife and handled with sterile forceps so as to prevent contamination. With the soft cheeses, the thoroughly ripened portion may be difficult to handle with a trier and a sterile spoon or spatula is advisable; care must be taken to keep away from the surface because of the abundance of organisms there.

A three inch mortar is a satisfactory size for use in grinding cheese for bacteriological analysis. The mortar and pestle may be wrapped together in paper and heated in a dry sterilizer. Fine sea sand makes a good grinding material but finely broken glass has also been employed; sterilization is conveniently carried out in a cotton stoppered test tube. Sucrose has occasionally been used for grinding and has the advantage of going into solution in the water blanks. It may be sterilized by flooding with ether for several days, after which the reagent is poured off and that then remaining evaporated.

By adding the grinding material to the mortar in small amounts the cheese can be more easily broken up than when it is added in one lot. The cheese must be very thoroughly divided and, with rapid work, this requires from 20 to 30 minutes' grinding. The appearance of the material in the water blank usually shows how completely the cheese has been disintegrated.

Beef infusion agar and whey agar are suitable plating media because most of the organisms in cheese are typical milk forms. Many of the types that are very numerous grow poorly on beef extract agar. A better colony development is secured at room temperature, 21° C. (69.8° F.) or 30° C. (86° F.), for from three to five days, than at 37° C. (98.6° F.) for a shorter period.

Because of the great difficulty of completely breaking up the colonies existing in a mass of cheese, the values obtained in determinations of the numbers of bacteria are regularly too low. However, results obtained under careful conditions on different lots or on the same cheese at different times show, in a general way, the changes in both the numbers of organisms and the important types. If reliable data are to be secured, the grinding of the samples must be thorough and uniform so that large clumps of bacteria will not be left undisturbed. Variations in the moisture content make it advisable, in certain investigations, to run moisture determinations on material similar to that used for the bacterial counts so that the results can be calculated to a moisture free basis.

For rough determinations of the numbers of certain organisms, such as *L. casei,* that develop poorly on plates but well in milk, a method consisting of putting dilutions of the ground-up cheese into tubes of sterile milk has been employed. The tubes are held for a time, usually one to two weeks, at a temperature favorable for growth and then examined in the gross and also microscopically in order to secure information on the types of organisms in the various dilutions. If growth occurs in a tube of milk inoculated with 1/500,000,000 of a gram of cheese but not in a tube inoculated with 1/1,000,000,000 of a gram, the number of organisms is between 500,000,000 and 1,000,000,000 per gram. If more accurate results are wanted, the amounts of cheese used can differ less and several tubes can be inoculated with each amount.

Valuable results on the micro-organisms in cheese can sometimes be secured by making direct microscopic examinations. A piece of cheese with a smooth surface may be pressed firmly against a clean slide and then removed so as to leave organisms distributed on the slide in much the way they were on the surface of the cheese. The "impression" is then fixed, freed from fat with some such solvent as xylol, stained and examined. The use of a direct microscopic examination with cheese should be as valuable as it is with other materials for the purpose of showing that important organisms are not being missed by the plate method.

Certain investigators have found it possible to cut sections of cheese for microscopic examination in the same way that sections of tissue are cut. Hucker[1] used such a procedure with cheddar cheese and found that the numbers of organisms determined by this direct examination were much larger than the numbers secured with the plate method.

BACTERIOLOGY OF CHEDDAR CHEESE

The normal ripening of cheddar cheese involves the action of several types of bacteria. These organisms multiply in the cheese and each type brings about a very definite change that is important from the standpoint of the ripening process. Other species of micro-organisms are also regularly present and, while certain of them are unimportant, others bring about undesirable conditions, such as the development of off flavors, gas formation, etc.

Type of milk desired. At the time the rennet is added to the milk, there should be large numbers of active *S. lactis* organisms present. This insures a rapid acid production during the manufacturing and the early stages of ripening, which is very necessary if the normal changes are to occur. The desired lactic acid bacteria may be secured by holding the milk for a short time at a temperature favorable for growth, but this also permits the development of objectionable species and the addition of a good culture commonly gives a more desirable flora in the coagulum.

---

[1] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 87. 1921.

Effect of coagulation with rennet on the distribution of organisms. When milk is coagulated with rennet, the majority of the contained organisms are carried into the curd so that the whey contains a smaller number of organisms per cubic centimeter than the milk from which it came. Hastings, Evans and Hart[2] have shown this by both laboratory trials and tests under practical conditions; representative results taken from their data are as follows:

COMPARISON OF BACTERIAL CONTENTS OF MILK BEFORE CURDLING AND WHEY IMMEDIATELY AFTER CURDLING

Laboratory Trials	Bacteria per CC.	
	Milk Before Curdling	Whey Immediately After Curdling
1	128,000,000	34,000,000
2	11,000,000	2,900,000
3	2,050,000	730,000
Tests under Practical Conditions		
1	5,600,000	3,000,000
2	6,400,000	1,290,000
3	5,430,000	2,400,000

These investigators found that, on the average, about 77 per cent of the organisms in the milk were held in the curd in the laboratory trials and about 73 per cent in the tests under practical conditions.

The concentration of organisms in the curd would be expected to result in a very rapid production of acid there since most of the organisms would be lactic acid types. Hastings, Evans and Hart[3] have presented data illustrating this; deep beakers were filled with milk, the milk curdled with rennet, the curd cut and allowed to settle and then the acidity of the whey at the top and bottom determined at various times, the beakers being held in a thermostat at 35° C. (95° F.) to eliminate convection currents. The findings in two of the trials are as follows:

---

[2] Wis. Agr. Expt. Sta. Res. Bul. 25. 1912.

[3] See ref. 2.

INFLUENCE OF THE CONCENTRATION OF ORGANISMS IN THE CURD ON THE ACID DEVELOPMENT IN WHEY

After	Acidity of Whey			
	Trial 1		Trial 2	
	Top	Bottom	Top	Bottom
0 hours........	0.09	0.09	0.14	0.14
2 hours........	0.09	0.10	0.21	0.34
4 hours........	0.11	0.13	0.32	0.64
6 hours........	0.12	0.22	0.36	0.71
8 hours........	0.16	0.49	0.56	0.80
10 hours........	0.27	0.67		
12 hours........	0.40	0.68		

The greater acid development at the bottom indicates that acid production is very rapid in the curd. This activity of the lactic acid organisms undoubtedly persists during the remainder of the handling of the curd.

Changes in the numbers of bacteria during the early part of the ripening. After the curd goes to press there is a further growth of the lactic acid organisms. Harrison and Connell,[4] employing the plate method with beef peptone gelatin or peptone whey gelatin, usually found the highest bacterial content in normal cheese when it was one day old, although occasionally the maximum occurred in from two to five days; the highest number of bacteria noted was 635,000,000 per gram. Lactic acid organisms were regularly the predominating type. Harding and Prucha [5] also secured data, with the plate method, indicating that the largest number of organisms occurs early in the ripening process; the maximum bacterial content noted for each of the nine cheese studied by them and the age at which it occurred are given in the following table.

---

[4] Ont. Agr. Col. Bul. 130. 1903.

[5] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 8. 1908.

Maximum Number of Organisms Noted in Cheese and the Age at Which It Occurred

Cheese	Maximum Number of Bacteria Found, per Gram	Age of Cheese When Maximum Number Was Noted, Days
1	135,700,000	4
2	69,600,000	4
3	79,200,000	2
4	37,100,000	10
5	90,600,000	2
6	106,300,000	8
7	26,800,000	1
8	51,000,000	25
9	177,900,000	1

These investigators reported that *S. lactis* was the only species which was always found and that it regularly made up over 99 per cent of the total bacterial content. Hastings, Evans and Hart[6] also found that the maximum number of bacteria, as measured by lactose agar plates, occurred early in the ripening process; in eight of the eleven cheese examined, the largest number of bacteria was found within 48 hours of making. A number of counts over 1,000,000,000 per gram was secured and the maximum was 2,518,000,000.

Because of the influence of various factors, there is undoubtedly considerable variation in the maximum number of organisms present in different lots of cheese and in the time this maximum occurs. Lactose greatly favors the growth of *S. lactis* and the amount of this retained is rather directly proportional to the moisture content of the cheese. It seems probable that, during the early ripening, the maximum number of organisms is reached at about the time the lactose disappears. The temperature of holding would be expected to have an influence on the rate of the growth of the organisms.

Following the increase in the organisms that are ordinarily determined by the plate method, there is a decrease which is rapid at first but later much slower. The decrease involves principally *S. lactis* and is due to the development of conditions that are unfavorable for the growth of this species. Occasionally, however, *S. lactis* is found in very old cheese.

---

[6] See ref. 2.

Importance of acid development. Under normal conditions *S. lactis* is the first organism to bring about an important change during the making and ripening of cheddar cheese and this consists primarily of a transformation of the lactose into lactic acid. The production of acid begins early in the making process and continues as long as lactose is available. This acid is very necessary for the proper changes during the manufacture and ripening; according to Hastings, Evans and Hart,[7] it (1) favors the curdling of the milk with rennet, (2) favors the expulsion of the whey, (3) favors the fusing of the curd particles, (4) has a protective action against putrefaction and (5) favors the action of the pepsin present in the rennet extract.

1. Favors the curdling of the milk with rennet. The activity of various enzymes is strikingly influenced by the reaction of the medium and, in agreement with this, more rapid coagulation of the milk with rennet is secured when there is a certain amount of developed acid present in the milk.
2. Favors the expulsion of the whey. The proper expulsion of the whey is necessary in order to reduce the moisture content of the cheese to a satisfactory point. If the moisture content remains too high the cheese is likely to have an off flavor and to fail to develop the desired condition. The rapid production of acid aids very materially in expelling the whey by causing a contraction of the curd. Sammis, Suzuki and Laabs[8] found that, with the percentages of acid in normal cheese curd, the expulsion of whey was proportional to the amount of acid present.
3. Favors the fusing of the curd particles. The fusion of the curd particles is necessary if the cheese is to have the proper body. These particles must lose their identity and form a compact mass, rather than show a tendency to retain their shape. The acid favors this fusion materially and, without it, the cheese is often crumbly.
4. Has a protective action against putrefaction. The restraining action of acid on the putrefactive organisms is well known and illustrated by many types of food products. In cheese, these organisms, although regularly present because of their presence in milk, are practically always prevented from growing, due largely to the acid. Babcock, Russell, Vivian and Hastings[9]

---

[7] See ref. 2.

[8] Wis. Agr. Expt. Sta. Res. Bul. 7. 1910.

[9] Wis. Agr. Expt. Sta. 18th An. Rpt. P. 162. 1901.

noted that, when the acid and the sugar from which acid might be formed were washed from curd, it rather quickly became a mass of decomposing material, as a result of the action of the putrefactive organisms.

5. Favors the action of the pepsin present in the rennet extract. Rennet extract contains the proteolytic enzyme pepsin in addition to the coagulating enzyme rennin. The pepsin plays an important part in the breaking down of the casein derivatives and its action is favored by the presence of the acid produced by the lactic acid organisms. This action can be readily observed by adding about two cubic centimeters of sterile milk to each of two tubes of agar, inoculating one with a very small amount of an *S. lactis* culture and then emptying each tube into a sterile petri dish. After incubating, so that the organisms in the one dish have had an opportunity to grow, a narrow strip of filter paper, moistened with rennet extract, is placed on each agar surface; both dishes are then held at 37° C. (98.6° F.). In a short time the dish in which *S. lactis* has produced acid will show a clear area around the paper, because the pepsin in the acid medium has changed the casein to soluble compounds, while the dish in which there has been no acid formation will show little if any clearing.

Growth of the lactobacilli. The lactobacilli constitute another type of organism bringing about changes that are important in the ripening of cheddar cheese. These organisms develop late in the ripening period and are present in very large numbers, from one to two billion per gram being commonly noted. The lactobacilli develop so poorly on agar that they are easily missed in plating out samples of cheese; growth on whey or infusion agar occurs when inoculations are made with a portion of a milk culture, but colonies often fail to develop when growth must start from a single cell or, at most, from a small clump. The method of adding dilutions of ground-up cheese to tubes of sterile milk is much more satisfactory than plating for the detection of the enormous numbers in which these organisms may be present in cheese.

The comparative numbers in which *S. lactis* and the lactobacilli are found in a cheese at different ages is illustrated by the following data taken from the results of Hastings, Evans and Hart:[10]

---

[10] See ref. 2.

Comparative Numbers in Which *S. lactis* and the Lactobacilli Were Found in a Cheese at Different Ages

Age of Cheese, Days	*S. lactis* Per Cent	Lactobacilli Per Cent
14	99.99	0.01
22	99.90	.10
37	90.90	9.10
45	99.90	.10
77	99.00	1.00
100	90.90	9.10
155	50.00	50.00
165	1.00	99.00
187	1.00	99.00

These results show very clearly that, from the standpoint of numbers, *S. lactis* is important early in the ripening period while the lactobacilli are dominant later.

Types of lactobacilli in cheese. Under the usual conditions, lactobacilli gain entrance to milk from a number of sources, such as feeds of various sorts and the intestinal tracts of the animals and, since the lactobacilli in cheese come primarily from those present in the milk, variations in the types found in cheese are to be expected. The studies that have been carried out show that differences occur in the morphology, the fermenting powers, the amount and isomeric form of lactic acid produced, etc. The lactobacilli so regularly found in cheese are often referred to as *L. casei,* although the relationship of the organisms in this group is not well understood.

Conditions making possible the growth of the lactobacilli in cheese. It should be noted that the growth of the lactobacilli in cheese occurs, for the most part, at a time when the lactose has been used up by *S. lactis;* presumably in cheese, as in milk and cream, *S. lactis* is capable of more rapid development than the lactobacilli so that the growth of the latter type is limited to a period when the conditions have become unsatisfactory for the former. The lactobacilli are favored by the presence of peptone and, since the changes occurring in the ripening of the cheese result in the formation of this product, it seems probable that the development of these organisms is favored by the peptone accumulation. The influence of peptone on the activity of the lactobacilli in such a satisfactory medium as milk is shown by the following data, taken from the results of

Hastings, Evans and Hart;[11] the acid production was used as a measure of the growth of the organisms.

INFLUENCE OF PEPTONE ON THE ACTIVITY OF LACTOBACILLI IN MILK

	Culture A		Culture B	
	Per Cent Acid in		Per Cent Acid in	
	10 Days	42 Days	10 Days	42 Days
Plain milk	1.24	1.40	1.58	1.93
Milk plus 0.5 per cent peptone	1.40	1.56	1.87	2.13
Milk plus 1.0 per cent peptone	1.58	1.77	1.96	2.25

The sequence of changes that appears to lead to the development of the lactobacilli is (1) the production of lactic acid by *S. lactis,* (2) the activity of pepsin as a result of the favorable reaction resulting from this acid, (3) the formation of peptone through the action of pepsin and (4) the stimulation of the lactobacilli by the peptone.

Changes produced in cheese by the lactobacilli. The most important change effected in cheese by the lactobacilli is in connection with the breaking down of the protein. These organisms increase the amount of soluble nitrogen in milk in which they grow and the same thing undoubtedly occurs in cheese. The proteolytic action of the lactobacilli is evident from the studies of various investigators; the following data are taken from those presented by Hastings, Evans and Hart[12] and involve an incubation period of three months.

THE PROTEOLYTIC ACTION OF THE LACTOBACILLI IN MILK

Culture Number	Grams Soluble N in 100 CC. of Milk	Increase in the Soluble N in 100 CC. of Milk Due to the Lactobacilli	
		Grams	Per Cent
Sterile milk	0.064		
113	0.076	0.012	18.7
120	0.081	0.017	26.5
116.2	0.092	0.028	43.7
52	0.104	0.040	62.5

---

[11] See ref. 2.

[12] See ref. 2.

These results show that the lactobacilli bring about pronounced increases in the soluble nitrogen in milk.

Additional organisms found in cheese. In addition to *S. lactis* and the lactobacilli, various organisms are found in cheese more or less frequently. Certain of these have a desirable influence on the ripening, others are inert, while still others are definitely objectionable because they induce abnormal conditions.

The streptococci other than *S. lactis* appear to be a group of importance in developing the flavor and aroma of cheese. Organisms of this general type are known to be necessary in the production of the desired flavor and aroma in butter. Evans[13] found two species of streptococci, which were designated *Streptococcus X* and *Streptococcus kefir,* common to ripening cheese of various kinds; these organisms and another unclassified Streptococcus, either singly or in combination, improved the flavor and hastened the softening of cheese of the cheddar type made from pasteurized milk. Hucker and Marquardt[14] found that *S. paracitrovorus* when added to milk, either alone or in conjunction with commercial starters, appeared to have a desirable effect on the flavor of cheddar cheese; with cheese made from pasteurized milk into which this organism had been inoculated, a flavor not unlike that of raw milk cheese developed. These investigators reported that *S. citrovorus* appeared to have no effect on the production of flavor in cheese made from milk to which it had been added. In the studies of Hart, Hastings, Flint and Evans,[15] both streptococci and micrococci isolated from cheddar cheese produced large quantities of volatile acid, especially acetic, when grown in milk; the milk used was practically free from fat so that the acids must have come from such sources as citric acid, lactose and protein. These cocci are at times present in cheese in large numbers so they were considered to be a factor in the volatile acid production that occurs during ripening.

Protein decomposition in cheese ripening. From the chemical standpoint one of the very important changes in the ripening of a cheese is the breaking down of the casein and casein derivatives. The tough and rubbery green curd must undergo extensive protein degradation before it becomes the type of product that is recognized as well-ripened cheese. This decomposition is effected in the main by enzymes and bacterial action; the enzymes involved are pepsin and galactase, while various organisms play a part.

---

[13] Jr. Agr. Res. 13. P. 235. 1918.

[14] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 117. 1926.

[15] Jr. Agr. Res. 2. P. 193. 1914.

During the early studies on cheese ripening there was considerable difference of opinion as to whether or not the rennet extract has an effect in addition to coagulating the milk. Trials at the New York (Geneva) Agricultural Experiment Station[16] indicated that with an increase in the amount of rennet used there was an increase in the soluble nitrogenous products formed. The results of Babcock, Russell and Vivian[17] show this same effect and also that the increase in the products formed is confined to the higher decomposition bodies that are peculiar to pepsin, thus suggesting that the digestive action of rennet extract is attributable to the pepsin incorporated with the rennet. In cheese made from milk to which purified pepsin had been added, either with or without the use of rennet to curdle the milk, the protein decomposition was increased. The American findings along this line are confirmed by the results secured in Europe.

An acid reaction is important in connection with peptic digestion and, in cheese, this is provided through the activity of *S. lactis.* Babcock, Russell and Vivian[18] found that acid salts, such as phosphates, favor peptic digestion in a manner comparable to free acids.

Galactase increases the amount of soluble nitrogen in milk held with a reagent, such as chloroform, to prevent bacterial growth and this suggests that it is important in the protein decomposition occurring during the ripening of cheese. Most of the investigations of this enzyme have been stimulated by the cheese ripening problem but the exact influence it has in a cheese is difficult to determine because of the activity of other proteolytic agents. With galactase the protein decomposition goes to the amino acid stage and considerable nitrogen in this form is one of the characteristics of ripened cheddar and other hard cheeses. It should be noted that the acid reaction in these cheeses is not as satisfactory for the activity of galactase as a neutral or slightly alkaline one.

Van Slyke, Harding and Hart[19] made cheese from milk to which from 4 to 5 per cent of chloroform had been added and then held the cheese, containing 12 to 15 per cent chloroform, in an atmosphere of chloroform. The soluble nitrogen formed was less than in normal cheese and the proportion of albumoses and peptones was high in comparison with the amides, while in the normal cheese the

[16] N. Y. (Geneva) Agr. Expt. Sta. Bul. 54. 1893.

[17] Wis. Agr. Expt. Sta. 17th An. Rpt. P. 102. 1900.

[18] See ref. 17.

[19] N. Y. (Geneva) Agr. Expt. Sta. Bul. 203. 1901.

amides were high. These results show the effect of the enzymes on the ripening process and also indicate that bacterial action is necessary for the normal changes. When 0.2 per cent lactic acid was added during the making of the cheese containing chloroform, the amount of soluble nitrogen was markedly increased, which suggests the importance of pepsin in the protein decomposition.

Among the organisms involved in the protein decomposition in cheese the lactobacilli are especially important, as has already been pointed out. Many of the *S. lactis* organisms, including those ordinarily present in cultures, also have the ability to increase the soluble nitrogen in milk and presumably they do the same thing in cheese. The growth of most of the typical proteolytic organisms that are so commonly present in milk is checked by acid but there may be some types that are active in cheese; these could be beneficial when present and satisfactory ripening still go on in their absence. *S. liquefaciens* has been suggested as of importance in cheese ripening but an extensive development of this type would seem undesirable because of the bitter flavor commonly developed.

In connection with the protein decomposition by *S. lactis* it is of interest to note that Barthel and Haglund[20] made cheese with a gouda texture from pasteurized milk, using cultures of *S. lactis* differing in protein decomposing powers, and found that the percentages of soluble and amino nitrogen in the various lots were essentially the same.

The general character of the protein decomposition at various stages in the ripening of cheddar cheese is illustrated by the following data presented by Van Slyke and Hart;[21] each analysis represents an average of results obtained under various conditions.

PROTEIN DECOMPOSITION DURING THE RIPENING OF CHEDDAR CHEESE

Age of Cheese, Months	Percentage of the Total Nitrogen in the Form of				
	Water Sol. N	Caseoses	Peptones	Amides	Ammonia
1½	21.44	3.15	3.84	9.88	1.56
3	30.98	4.56	4.65	14.36	2.45
6	36.15	4.92	4.22	19.96	3.52
9	43.45	4.59	3.56	26.53	4.74
12	44.75	4.16	3.95	28.38	5.41
18	47.25	3.88	2.57	30.46	6.62

---

[20] Proc. World's Dairy Cong. 1. P. 314. 1923.

[21] N. Y. (Geneva) Agr. Expt. Sta. Bul. 236. 1903.

The data show that even with extended ripening less than half of the nitrogen is in a water soluble form and that amides make up a considerable portion of the water soluble nitrogen. This type of protein decomposition is more or less characteristic of the hard cheeses generally.

The lactic acid in cheese. During the early part of the ripening of a cheese lactic acid is rapidly developed, principally through the action of *S. lactis.* Suzuki, Hastings and Hart[22] found that the amount of lactic acid fluctuated during the ripening process but that some remained, even after an extended curing period; the general change in this constituent is shown in the following tabulation of their data.

LACTIC ACID IN CHEESE AT VARIOUS AGES

Age of Cheese	CC. of n/10 Lactic Acid per 100 Grams of Dry Cheese	
	Whole Milk Cheese	Skim Milk Cheese
3 days........	84.09	149.77
6 weeks.......	90.28	119.60
3 months.....	124.00	179.44
5½ months....	103.70	132.30
10½ months....	74.10	11.60

The lactic acid that developed long after the lactose had disappeared may have been of protein origin. These investigators noted that the usual form of lactic acid in cheddar cheese is inactive; this may be due to the activity of the organisms present in the culture used since Hammer[23] has shown that such cultures may contain considerable amounts of inactive lactic acid. The disappearance of lactic acid is presumably due to its transformation to other compounds through the activity of organisms.

Changes in the volatile acidity during ripening. There appears to be first a rise and then a fall in the volatile acidity during the ripening of cheddar cheese. This is illustrated by the following data taken from the results of Suzuki, Hastings and Hart.[24]

---

[22] Wis. Agr. Expt. Sta. Res. Bul. 11. 1910.

[23] Ia. Agr. Expt. Sta. Res. Bul. 65. 1920.

[24] See ref. 22.

Volatile Acidity in Cheese at Various Ages

Age of Cheese	CC. of n/10 Volatile Acid per Gram of Dry Cheese	
	Whole Milk Cheese	Skim Milk Cheese
3 days........	12.74	21.60
6 weeks.......	34.17	62.29
3 months.....	32.15	105.71
5½ months....	34.82	84.20
10½ months....	22.96	

These investigators found that acetic and propionic acids reached a maximum at three months and then decreased, while butyric and caproic acids increased continuously during the period of study. Formic acid was found once, at 5½ months, in the whole milk cheese and not at all in the skim milk cheese. Valerianic acid was never obtained. Lactates were probably the principal source of acetic and propionic acids while the fats and proteins were the source of butyric and caproic acids. Hammer[25] has shown that the volatile acid production of *S. citrovorus* can be increased by adding sterile lactic acid to the milk in which the organism is grown.

Alcohols and esters from cheese. Suzuki, Hastings and Hart[26] distilled ground cheese with steam after moistening it with dilute H_2SO_4 and then redistilled after neutralizing with $Ba(OH)_2$. The final distillate was characterized by an odor resembling that of cheese and contained alcohols and esters; from whole milk cheese the esters were largely of ethyl alcohol and acetic acid, while from the skim milk cheese they were largely of ethyl alcohol and caproic and butyric acids.

Succinic acid in cheese. Succinic acid was isolated from ripening cheese by Suzuki, Hastings and Hart[27] and identified by its silver salt. It was also secured from a solution of lactose and peptone, fermented in the presence of $CaCO_3$ and fat, by the inoculation of a small bit of cheese.

---

[25] Ia. Agr. Expt. Sta. Res. Bul. 63. 1920.

[26] See ref. 22.

[27] See ref. 22.

Defects of Cheddar Cheese Due to Micro-organisms

A process that involves the extended growth of a few desirable types of organisms from a flora as varied as that ordinarily found in milk, may be expected to show considerable irregularity. Defects in cheese due to micro-organisms are, accordingly, rather common; certain of these result from the activity of undesirable species and others are due to the failure of the desirable types to develop normally. Some of the defects are so slight they are of little importance while others are very objectionable and seriously lower the value of the cheese.

Quality of milk for cheese. If milk intended for cheesemaking contains a variety of organisms in large numbers, types that are capable of producing undesirable changes are quite certain to develop under the conditions provided. The necessity for the growth of desirable organisms implies that there is the possibility of the development of objectionable types, if they are present, and the manufacture of cheese requires milk of a good quality, bacteriologically. Some of the manufacturing and curing methods tend to minimize the action of these undesirable organisms; for example, the salt added may restrain the development of certain species.

The total number of bacteria is not a satisfactory measure of the quality of milk for cheesemaking. Large numbers of lactic acid bacteria are desirable while comparatively small numbers of some type capable of bringing about objectionable changes may be very serious. Investigations carried out by Hucker[28] showed no striking relationship between the number of bacteria in the milk, as determined either by direct counts or by using the acidity as an index, and the score of the cheese, although it was noted that milk containing from 12 to 42 million bacteria per cc. produced cheese of more constant quality than did milk containing a smaller number.

Lack of proper acid development. A comparatively slow acid development sometimes occurs during the manufacturing process. The reasons for this are often difficult to determine; an inactive culture is a common cause but, in some instances, it appears that the lactic acid organisms are restrained by other types present. With this slow acid development large amounts of moisture and materials in solution in it are retained and these afford an unusual opportunity for the development of objectionable organisms. An excessively acid flavor more or less regularly occurs in cheese having a high moisture content because of the abundance of lactose available, but other off flavors may also appear through the action of an abnormal flora. The failure of the typical acid development to occur seriously influences the texture of the cheese.

---

[28] N. Y. (Geneva) Agr. Expt. Sta. Bul. 486. 1921.

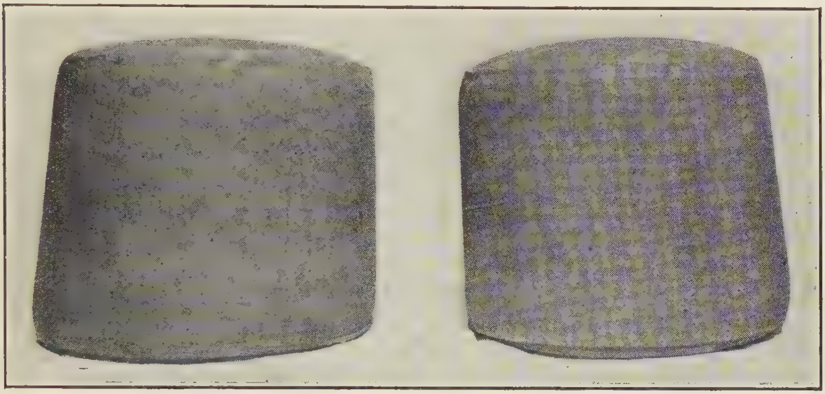


FIG. 41.—Cheddar cheese showing gas formation.

Gassy or swelled cheese. The formation of gas holes is one of the cheese defects commonly noted. Usually it occurs early in the curing period but occasionally there is a late development. Under normal conditions some gas is produced in a cheese but this develops so slowly that it has an opportunity to diffuse out as fast as it is formed and gas holes result only when excessive amounts are produced. Van Slyke and Hart[29] found that, during the ripening of

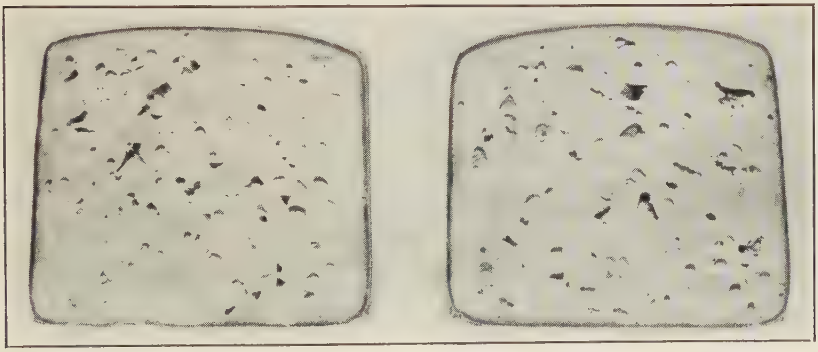


FIG. 42.—Gas holes in cheddar cheese.

---

[29] N. Y. (Geneva) Agr. Expt. Sta. Bul. 231. 1903.

a normal cheese, carbon dioxide was given off continuously, although in decreasing amounts after about 20 weeks. The total amount produced was 15.099 grams, equivalent to 0.5 per cent of the fresh cheese. In a cheese made from milk containing chloroform and kept under antiseptic conditions, the total amount of carbon dioxide produced was 0.25 gram, practically none of which was formed after three weeks. These investigators considered that, with the normal cheese, the carbon dioxide given off early in the ripening came from the decomposition of lactose by lactic acid organisms, while a small amount was probably due to the gas present in the milk and to the respiration of the organisms in the cheese. The carbon dioxide produced later apparently came from reactions taking place, among which was identified the change of tyrosin and arginine to derived products.

Russell[30] did some of the early American work on the formation of gas in cheddar cheese. He isolated a number of organisms producing gas in milk and found, through inoculation experiments, that all of them had a marked effect on the texture of the cheese, making it more or less spongy, and that the flavor was invariably poorer than the flavor of the controls. In one trial the development of gas was so pronounced that the cheese huffed into a football shape and rolled off the shelf. The gas forming organisms isolated apparently belonged to the Escherichia-Aerobacter group.

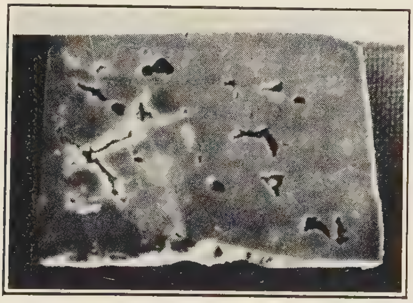


FIG. 43.—Cheddar cheese showing mechanical and gas holes. The gas holes resemble those normally present in Swiss cheese. Courtesy G. Wilster.

A gassy condition in cheese, which was accompanied by a definite off odor, was investigated by Moore and Ward[31] and found to be due to an organism belonging to the Escherichia-Aerobacter group. In the attempts to locate the source of the organism, milk drawn directly into sterilized jars under very careful conditions was found to contain it; this occurred both with the fore milk and with that coming from later in the milking. Udder infections with organisms of this type appear, however, to be rather unusual.

[30] Wis. Agr. Expt. Sta. 12th An. Rpt. P. 139. 1895.
[31] N. Y. (Cornell) Agr. Expt. Sta. Bul. 158. 1899.

Marshall[32] investigated cheese curd showing a marked inflation in the vat and isolated an organism which caused an extensive gas development in cheese when it was added to the milk used in the manufacture; it apparently belonged to the Escherichia-Aerobacter group.

In an investigation of gas producing bacteria and their effect on milk and its products, Harrison[33] found that the milk from 2 of 25 cows contained the organisms, even with every possible precaution to guard against contamination from the air and the coat of the cow and concluded that, occasionally, gas formers come from the interior of the udder; other sources were found to be the coat of the cow, the milk cans, the watering trough, flies and manure. When the organisms isolated were inoculated into milk, the cheese made from it was typically gassy, the flavor and aroma were very objectionable and there was a mottled appearance which was thought to be due to the action of the gas. Trials showed that the gas formers varied in the way they were restrained by cultures.

The organisms responsible for gas in cheese seem to very commonly belong to the Escherichia-Aerobacter group. In some instances, however, the lactose fermenting yeasts have been involved and in others the spore forming anaerobes have caused the condition. A rapid acid development during the making of cheese tends to restrain the gas formers of the Escherichia-Aerobacter type but may not entirely prevent their activity.

Off flavors in cheese. Off flavors in cheese may be due to any one of a large number of organisms that occasionally are present in sufficient numbers to play a part in the changes that occur. Bitterness is due especially to organisms that effect a certain type of protein decomposition. A putrefactive condition likewise results from protein decomposition in which the products have very objectionable flavors and odors; the responsible organisms seem to be favored by the absence of a proper acid development. A burnt or malty flavor, due to the development of *S. lactis var. maltigenes,* is sometimes encountered and may be very pronounced. Harding, Rogers and Smith[34] investigated a class of off flavors designated as "sweet" and concluded that they were due to yeasts. Normal flavored cheese contained very few if any yeasts while cheese showing the sweet flavor contained large numbers, at least during the first stages of ripening.

---

[32] Mich. Agr. Expt. Sta. Bul. 183. 1900.

[33] Ont. Agr. Col. Bul. 141. 1905.

[34] N. Y. (Geneva) Agr. Expt. Sta. Bul. 183. 1900.

The addition of cultures of certain yeasts to the cheese vats repeatedly caused off flavors in the cheese, some of which would be included under the general term of sweet flavor.

It appears that almost any of the off flavors which develop in milk or cream may also be found in cheese. The composition of the product may be a factor in the development of certain off flavors; for example, experiences in various cheese factories indicate that fruity flavors in cheese are most common when the moisture is high and the acidity low.

An outbreak of bitterness in cheese has been described by Harrison.[35] The bitter condition was occasionally noted when the milk was dumped into the weigh can but was more evident when the milk was warmed up for setting and the odor increased as the curd was heated. When the curd was very bitter the top of the whey showed small gas bubbles, especially on heating. The bitter flavor was permanent in the cheese. There was a variation from day to day in the extent of the bitterness and it was sometimes accompanied by an aromatic or fruity flavor. A yeast which produced a bitter taste in milk was isolated from samples of bitter curd. Other yeasts were also present, some of which gave pleasant, ethereal odors in beer wort. Samples of milk were taken from individual farms and, of 99 samples from as many patrons, yeasts were found in 97, in many cases several species being present; the predominant variety was the one giving the bitterness in milk and it was named *Torula amara.* The organism was repeatedly isolated from can washings and whey and was also found on the leaves of certain maples. Ten minutes at 62° C. (143.6° F.) killed it but it was not destroyed in a can when the can was scalded with water at about 93.3° C. (200° F.). Gas was produced in milk. In 5 or 6 hours at 37° C. (98.6° F.) inoculated milk had a bitter flavor and after 14 hours the flavor and aroma were disagreeable and strong; in 10 days at 37° C. the milk was thickened and slightly acid. Butter made from cream into which the organism had been inoculated showed a pronounced, bitter, disagreeable flavor.

Color changes in cheese. The color changes in cheese, as a result of the activity of micro-organisms, consist especially of the development of red or pink areas in the cheese mass and of the growth of molds.

Harding and Smith[36] studied the development of red or rust colored spots in the cheese made in a number of New York factories and found that the organism responsible in all cases was one which Connell[37] had isolated in Canada and described as *Bacillus rudensis.* The spots were very fine and were quite evenly distributed throughout the cheese, although they were most noticeable on the surface of the gas holes and other openings; from four to eight days were usually required for their development. The texture and flavor did not seem to be influenced by the development of the color. Although cheese made from the milk of individual patrons indicated that the milk coming to the plant at times contained *B. rudensis,* the factory contamination appeared to be the main source of the organism. Thorough steaming of all the utensils and equipment, together with good factory sanitation, made it possible to control this abnormality. The return of whey to the farms may have been a means of scattering the organism in the barn surroundings.

---

[35] Ont. Agr. Col. Bul. 120. 1902.

[36] N. Y. (Geneva) Agr. Expt. Sta. Bul. 225. 1902.

Mason[38] has reported results on the discoloration of cheese made on the Wensleydale system that are of interest in this connection. The cheese was normal in appearance when first cut but became pink soon afterward, the color appearing along the fine cracks and veins in the cheese. The color penetrated only to a depth of about 0.5 mm. and the tinted portion could be sliced off, leaving a normal surface that in turn became pink. A yeast was isolated which gave a pink color in various media. By adding the organism to milk, a pink color was produced in cheese made so that the curd was soft; in cheese made on the cheddar system no pink color resulted, probably because of the texture of the curd.

Cheese provides materials that are very satisfactory for the nutrition of molds and, since spores are quite regularly present, the development of these organisms occurs when the requirements as to air, humidity and temperature are met. Because of the air supply necessary, mold growth occurs mainly at the surface of a cheese; it may be largely controlled here by a good coat of paraffin but cracks in the paraffin, which are often due to the collection of moisture beneath it, afford an opportunity for growth to start and it may then extend rather rapidly. In some instances mold growth follows openings down into the cheese and the plugging of cheese commonly results in the development of molds along the plug. The humidity and temperature of the curing and storage rooms for cheese are commonly satisfactory for mold growth so that the air supply is the principal controlling factor.

---

[37] Canadian Dept. of Agr. Bul. 1897.

[38] Jr. Ministry Agr. 27. P. 520. 1920.

The contamination of the surface of cheese with molds, as a result of their growth in the curing room, sometimes occurs; this is especially serious when molds are growing on the shelves used for holding the cheese but may also result through air contamination.

Manufacture of cheddar cheese from pasteurized milk. The wide use of pasteurization in the manufacture of butter suggested this process as a means of controlling the abnormal fermentations in cheddar cheese. In certain countries, especially New Zealand, it has been extensively practiced and the cheese made has proved satisfactory on the important markets of the world. Stevenson[39] reported that while prior to 1914 the manufacture of cheddar cheese from pasteurized milk was practically unknown in New Zealand, in 1923, 38,000 tons or approximately two-thirds of the output of the Dominion was made by this system. The method involved flash heating to from 71.1° to 73.9° C. (160° to 165° F.) with a regenerative system, the use of from one to one and one-fourth per cent culture and an increase in the amount of rennet.

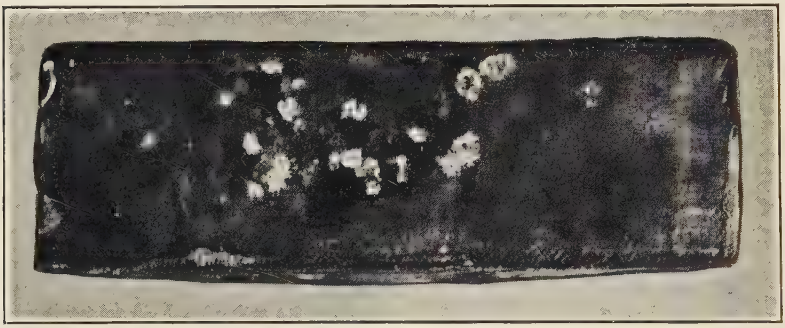


FIG. 44.—Cheddar cheese on which molds have begun to develop. Courtesy G. Wilster.

Sawers[40] has recently pointed out that the pasteurization of milk made into cheese in New Zealand has resulted in a product of a higher quality, which maintains its quality very much better under storage conditions than cheese made from raw milk and which also stands transit better. The exposure suggested for pasteurization is from 71.1° to 73.9° C. (160° to 165° F.) flash; if a lower temperature is used not all of the objectionable organisms will be destroyed, while if a higher one is employed the curd obtained will lose its cohesive properties.

---

[39] Proc. World's Dairy Cong. 1. P. 306. 1923.

[40] Jr. Dept. Agr. of Victoria. 23. P. 421. 1925.

In the United States some of the early work on the pasteurization of milk for cheddar cheesemaking was carried out by Sammis and Bruhn;[41] the method involved heating to 71.1° to 73.9° C. (160° to 165° F.) by the flash method and, after cooling, the addition of hydrochloric acid to restore the coagulability of the milk. Price[42] found that pasteurization at 62.8° C. (145° F.) for 30 minutes produced better cheese than the other methods of pasteurization tried; the procedure involved the addition of sufficient culture to produce 0.165 per cent acid in the whey at the time of dipping and sufficient rennet to give a curd firm enough to cut in from 15 to 20 minutes. The pasteurization was most effective when the raw milk was of an inferior quality but the quality of pasteurized milk cheese varied with the quality of the raw milk. Price and Prickett[43] investigated the effect of pasteurization of the milk by various methods on the quality of cheese, using (1) raw milk, (2) milk flash pasteurized at from 71.1° to 73.9° C. (160° to 165° F.) and cooled immediately by means of a surface cooler, (3) milk flash pasteurized at 62.8° to 65.6° C. (145° to 150° F.) and conducted to a vat where it was not cooled until the entire lot had been heated and (4) milk pasteurized at 62.8° C. for 30 minutes. The bacterial efficiencies obtained, as an average of 10 runs, with the various methods of pasteurization were 98.31 per cent for the flash method, 98.65 for the flash-holder method and 98.96 for the holder method. Pasteurization by any of the methods improved the quality of the cheese but the flash method was not as effective as the other two. The yield of cheese from pasteurized milk was from 2.5 to 4 per cent greater than the yield from identical milk not pasteurized, due to the retention of more moisture.

Cultures are regularly used in the making of pasteurized milk cheese because of the importance of the culture organisms in the ripening process. Some of the lactobacilli survive pasteurization so that, with the usual contamination of the milk, this type may be expected in the cheese. Investigations have been carried out in which different combinations of organisms have been added to the pasteurized milk to be made into cheese and some of these have given

[41] Wis. Agr. Expt. Sta. Res. Bul. 27. 1912.

[42] Jr. Dairy Sc. 10. P. 155. 1927.

[43] Jr. Dairy Sc. 11. P. 69. 1928.

a very satisfactory product. It seems probable that the flavor and aroma of high quality cheese may be due to the activity of combinations of organisms and that such combinations are not always readily established.

Clarification of milk for cheesemaking. The removal of most of the dirt and some of the bacteria from milk by clarification has suggested the use of this process with milk intended for cheese. Fisk and Price[44] found that clarification improved the quality of cheese, as compared to the quality of a product made from unclarified milk from the same lots, and the cheese kept better in storage. Clarification resulted in an improvement in the quality of cheese regardless of the quality of the milk from which it was made. The results reported by these investigators were secured in a number of different plants, both with and without the use of a culture.

Combs, Martin and Hugglar[45] concluded that the clarification of milk of a poor quality under ordinary plant conditions gave no increase in the score of cheese, while when extreme care was used in the plant there was an increase of 1.59 points; with milk of a high quality clarification under ordinary plant conditions resulted in an increase of 2.53 points, while when extreme care was used in the plant there was an increase of 2.83 points.

BACTERIOLOGY OF SWISS OR EMMENTAL CHEESE

Swiss or Emmental cheese is a typical hard variety that is characterized by gas holes or eyes, a sweetish flavor and a firm elastic texture. The general ripening process is comparable to the ripening of cheddar cheese, although the formation of eyes is the result of a change that is normal only to Swiss.

General morphologic groups of bacteria involved. Eldredge and Rogers[46] have studied the changes in the general morphologic groups of bacteria during the ripening of Swiss cheese by plating on a special beef extract peptone whey agar and, at each plating, picking all the colonies from a representative plate. Over 900 cultures were studied in detail. Fermentation tests failed to divide them into distinct groups but they were sharply differentiated morphologically into cocci, short rods and long rods. The young cheese contained short rods almost exclusively, but these decreased slowly while the long rods increased. After from six to eight weeks the two groups were present in about equal numbers while at the end of the ripening the long rods made up most of the flora. The cocci appeared in small numbers at intervals in the earlier stages of the ripening. The rods included typical cultures of lactobacilli but there were all gradations between these and cultures producing no acidity in milk.

---

[44] N. Y. (Cornell) Agr. Expt. Sta. Bul. 418. 1923.

[45] Jr. Dairy Sc. 7. P. 524. 1924.

[46] Centbl. f. Bakt. 2 Abt. 40. P. 5. 1914.

Protein decomposition. The agents that are responsible for protein decomposition in Swiss cheese are the same as those involved in cheddar cheese. The relationship between them may be somewhat different because of differences in the methods of manufacturing the two varieties of cheese, but the general type of decomposition is the same.

Eye formation. Eyes that are of the proper size and well distributed are very important in a Swiss cheese. Normal eyes vary in diameter from about one-half to one and one-fourth inches and are so spaced that there is from two to several inches between them. Under abnormal conditions the eyes may be small and numerous or large and few in number, while in some instances eye formation fails to occur. The ability of various bacteria to produce gas suggests that the eye formation is due to bacterial activity.

Clark [47] has shown that the gases of the normal eyes of Swiss cheese are carbon dioxide and nitrogen; the nitrogen is that of the air occluded in the curd at the time of making so the carbon dioxide is the only gas of significance. In some instances this investigator noted an evolution of gas characterized by the presence of hydrogen and considered it to be due to the formation of gas from the sugar; this type of gas production occurs soon after the cheese is made and is detrimental to it.

Because of the uneven distribution of bacteria in cheese it might be assumed that the eyes form at the location of colonies of the gas producing organisms. The comparatively few eyes in a normal Swiss cheese, however, make this improbable and, moreover, the types of organisms present near the eyes appear to be the same as those at a distance. Clark [48] has pointed out that there is no need to assume that the gas separates as a bubble at the point where it is produced and has shown that in agar or gelatin gas diffuses considerable distances. He succeeded in increasing the size of a gas bubble blown in agar by growing gas producing organisms some distance from it; in certain of the trials the gas diffused through a collodion sac in its

[47] U. S. D. A. B. A. I. Bul. 151. 1912.

[48] Jr. Dairy Sc. 1. P. 91. 1917.

passage from the region of bacterial growth to the point where it appeared as a bubble. Cultures of gas forming organisms in agar or gelatin containing a fermentable material commonly illustrate this gas diffusion; bubbles are not formed around each colony and colonies are often found at considerable distances from bubbles. Clark concluded that the gas in Swiss cheese separates at points that have no necessary relation to the localities where the gas is produced. When the curd particles were stained with Congo red by the addition of this dye to the kettle just before the curd was drawn, it was found that the interior walls of normal eyes were stained, which led to the assumption that the eyes developed between the curd grains.

Organisms responsible for eye formation. von Freudenreich and Orla-Jensen [49] isolated propionic acid producing bacteria which they considered the cause of the eye formation in Swiss cheese. The essential organism was found to ferment lactates with the production of propionic acid, acetic acid and carbon dioxide and was named *Bact. acidi-propionici* (*a*). From trials involving the addition of dilutions of cheese to calcium lactate broth, these investigators concluded that the organisms occur in cheese in numbers from 10,000 to 200,000 per gram.

Sherman [50] isolated a propionic acid producing organism from Swiss cheese and considered it to be the essential organism for the production of eyes and the characteristic flavor. It fermented lactates with the production of carbon dioxide and volatile acids, especially propionic and acetic acids; lactose also yielded carbon dioxide and volatile acids. Using a sodium lactate broth containing dried yeast, this investigator found that all of 16 samples of American made Swiss, which varied in flavor from excellent to very poor but each of which showed eyes, contained a lactate fermenting organism in numbers exceeding 1,000,000 per gram; the organism was recognized through its ability to produce volatile acid in the medium employed. Sherman concluded that he found the organism in larger numbers than von Freudenreich and Orla-Jensen because the medium he used was more favorable. The organism was easily isolated by adding dilutions of cheese to agar made with peptone, dried yeast and sodium lactate and then sealing the agar in glass tubes. Sherman considered that the organism isolated by him agreed closely with the *Bact. acidi-propionici* (*a*) described by von Freudenreich and

---

[49] Landw. Jahrb. Schweiz. 20. P. 320. 1906.

[50] Jr. Bact. 6. P. 379. 1921.

Orla-Jensen but showed some variations from it. He tentatively suggested the name *Bact. acidi-propionici (d).*

Shaw and Sherman[51] found that, in addition to fermenting lactose and lactic acid, *Bact. acidi-propionici (d)* produces carbon dioxide and propionic and acetic acids from succinates, glycerol, peptone and perhaps to a slight degree from butter fat; peptone gives a greater proportion of acetic to propionic acid than the other substances attacked. Aspartic acid was shown to serve as a source of carbon dioxide and acetic acid.

Considering the materials from which the eye forming organism can produce carbon dioxide and volatile acids, it would be expected that growth would be greatly influenced by the activity of other species in making such materials available. The following data, which have been presented by Sherman and Shaw,[52] show the propionic acid production of *Bact. acidi-propionici* (*d*) alone and in combination with various organisms; growth was for one month at 30° C. (86° F.) in a medium containing 5 per cent lactose, 1 per cent peptone and an excess of $CaCO_3$.

INFLUENCE OF ASSOCIATIVE ACTION ON THE PRODUCTION OF PROPIONIC ACID BY *Bact. acidi-propionici* (*d*) IN A LACTOSE PEPTONE BROTH WITH $CaCO_3$

Organisms Used	Per Cent Propionic Acid
Bact. acidi-propionici (*d*) alone	0.081
Bact. acidi-propionici (*d*) +*S. lactis*	0.470
Bact. acidi-propionici (*d*) +*L. casei*	0.589
Bact. acidi-propionici (*d*) +non-lactose fermenter	0.559
Bact. acidi-propionici (*d*) +non-lactose fermenter	0.362

From these results it is evident that in mixed cultures much larger amounts of propionic acid were produced than when *Bact. acidi-propionici* (*d*) was in pure culture; the differences can not be accounted for through the development of propionic acid by the organisms other than *Bact. acidi-propionici (d)* and are undoubtedly due to associative action.

[51] Jr. Dairy Sc. 6. P. 303. 1923.

[52] Jr. Gen. Physiol. 3. P. 657. 1921.

Defects in Swiss Cheese Due to Micro-organisms

Lack of eye formation. Swiss cheese sometimes fails to develop eyes, due to the absence of the eye producing organism or a limited growth of it; this organism should produce gas in amounts sufficiently large so that some of it comes out of solution in the cheese. The use of a culture of the eye forming organism in the milk to be made into cheese has proved very helpful in bringing about the desired condition.[53]

Gassy Swiss cheese. In some instances there is the production of excessive amounts of gas and, instead of the desired eye development, the cheese shows large numbers of gas holes; often the shape of the cheese becomes very abnormal. Gas formation in Swiss cheese may be the cause of serious economic losses; it sometimes occurs soon after the cheese is made, while in other cases it is delayed. The responsible organisms are of various types.

Russell and Hastings [54] investigated an outbreak in a Wisconsin factory and found the trouble due to a lactose fermenting yeast. When a culture of the organism was added to milk that was made into Swiss cheese it caused an extremely gassy condition and the cheese was decidedly lacking in flavor. These investigators pointed out certain practices at one time commonly used that tend to favor the growth of the organism; these are (1) holding the whey so that the butter fat can be skimmed off, (2) the use of whole rennets soaked in sour whey and (3) the use of individual barrels, which are rarely cleaned, to hold the whey for the various farmers.

Burri [55] reported that an excessive number of large holes and a splitting of the rind, together with an objectionable flavor and aroma, were due to large numbers of butyric acid organisms that came from silage. The beginning and ending of the abnormality in the cheese coincided so definitely with the beginning and ending of the silage feeding that the relationship was very clear.

Albus [56] investigated the cause of unusually large numbers of gassy cheese at a factory and, after failing to isolate a gas forming organism that would grow aerobically, secured a strain of *Clostridium welchii* that was responsible for the abnormal condition. Experimental cheese showed both the niszler type of gassy fermentation, which is characterized by a uniform distribution of very small holes that are detected when the cheese has been made for some time, and the pressler type, in which the gassy condition is noted on the press. In a few instances the cheese made from milk inoculated with the isolated culture of *C. welchii* showed little or no gas, although the usual method of manufacture was used. The results obtained suggest that some undetermined factor or group of factors plays an important part in the development of the gassy condition.

---

[53] Proc. World's Dairy Cong. 1. P. 287. 1923.

[54] Wis. Agr. Expt. Sta. Bul. 128. 1905.

[55] Proc. World's Dairy Cong. 1. P. 278. 1923.

[56] Jr. Bact. 15. P. 203. 1928.

Abnormal flavors and aromas. Swiss cheese sometimes develops abnormal flavors and aromas and these may suggest an objectionable type of protein decomposition. American factories have occasionally experienced an undesirable fermentation in which the cheese are referred to as "stinkers." Protein decomposition is more likely to occur in Swiss than in cheddar cheese because of the comparatively sweet character of the curd. Burri [57] reported an objectionable flavor and aroma associated with the development of a white crumbly condition and large irregular holes in parts of the cheese; *Bacillus putrificus* was found present in this material.

Control of gassiness by cultures of lactobacilli. The prominence of the lactobacilli in Swiss cheese led to the suggestion that a culture of one of these organisms be used in the manufacture of this product. After various studies in Europe, Doane and Eldredge [58] carried out some trials at various places in the United States with the special object of suppressing gas forming organisms. It was found that different cultures of *L. bulgaricus* varied greatly in their effect and, while several prevented gas formation when 2 per cent of a milk culture was added to the milk, others did not prove efficient with less than 4 per cent. Cultures of *S. lactis* did not prevent gas formation in the trials made. Under commercial conditions good Swiss cheese was produced at a season when it was not possible to make marketable cheese without the use of lactobacilli.

BACTERIOLOGY OF ROQUEFORT CHEESE

Roquefort cheese is a representative of the group of hard cheeses in which there is normally a mold growth in the cheese mass during the ripening period; these varieties are sometimes referred to as the blue veined cheeses. The mold is very active in bringing about changes and has a pronounced influence on the flavor and aroma development. Roquefort cheese was originally made in France from sheeps' milk. The Italian Gorgonzola and the English Stilton and Wensleydale are other important cheeses of this type.

---

[57] See ref. 55.

[58] U. S. D. A. Bul. 148. 1915.

S. lactis in Roquefort cheese. In the making of Roquefort cheese a development of acid is necessary and this occurs through the fermentation of the lactose by *S. lactis*. The addition of the usual type of culture to the milk assures the presence of this organism in satisfactory numbers. Thom, Currie and Matheson [59] have pointed out that the lactobacilli are not a factor in the initial acid formation. If acid does not develop properly the cheese fails to drain satisfactorily and the development of objectionable bacteria frequently occurs. After reaching large numbers, *S. lactis* soon dies out. Evans [60] found that it disappeared from cheese in about two or three weeks and ascribed this rapid destruction to the high concentration of salt.

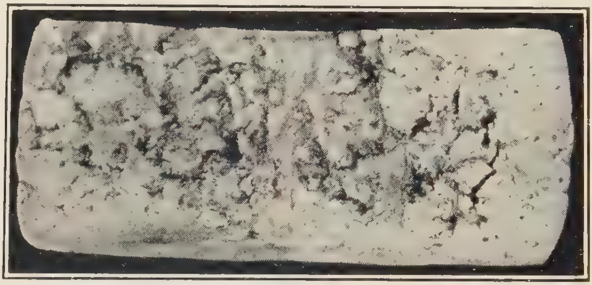


FIG. 45.—Normal mold development in Roquefort cheese.

Bacteria other than S. lactis. A number of bacterial species other than *S. lactis* have been found in Roquefort cheese by various investigators. Thom, Currie and Matheson [61] reported the presence of lactobacilli, liquefying organisms and yeasts while Evans found lactobacilli and streptococci. In the Stilton cheese investigated by Percival and Mason [62] a spore former was regularly present but never abundant.

In general, it appears that *S. lactis* is the only necessary bacterial species in Roquefort cheese. The presence of other organisms is to be expected, because of their existence in milk and their ability to survive the conditions in the cheese; while they may develop actively, the changes brought about by them are not vital to normal ripening.

---

[59] Conn. (Storrs) Agr. Expt. Sta. Bul. 79. 1914.

[60] Jr. Agr. Res. 13. P. 225. 1918.

[61] See ref. 59.

[62] Jr. Agr. Sc. 5. P. 222. 1913.

The mold of Roquefort cheese. The typical mold of Roquefort cheese has been named *Penicillium roqueforti* by Thom.[63] In some of the older literature the mold in the blue veined cheeses is referred to as *Penicillium glaucum*[64] but it is very probable that more than one species has been included under this name. *P. roqueforti* is by no means limited in its development to blue veined cheeses and is frequently found in other types of dairy products. It has been isolated from silage and is undoubtedly more or less regularly present in the stable surroundings.

The dominance of *P. roqueforti* in Roquefort cheese, when spores and vegetative growth of a variety of other molds are present as a result of the contamination during the production and handling, presents a problem in the relationship of this organism to the environment. Thom and Currie[65] analyzed the gas in both imported and experimentally made cheese and found the percentage of free oxygen low, 7.00 per cent being the highest value in five analyses. The carbon dioxide varied from 21.14 to 40.95 per cent; the percentage appears to be highest in comparatively young cheese and to fall off through diffusion as the cheese ages. These investigators studied a number of species of Penicillium and Aspergillus and noted that *P. roqueforti* tolerated a high percentage of carbon dioxide and a low percentage of oxygen better than any of them. *O. lactis,* however, can also grow at a low oxygen tension. Thom, Currie and Matheson[66] found this species would develop in Roquefort cheese when the salt concentration was 2 per cent but all the abnormalities due to it disappeared when a salting process giving 4 per cent salt in the newly made cheese was used. Accordingly, it appears that mold growth in Roquefort cheese is largely limited to *P. roqueforti* because it tolerates both a low oxygen tension and a high concentration of salt while other molds do not.

Changes produced by P. roqueforti. *P. roqueforti* brings about three distinct changes in a Roquefort cheese, (1) the destruction of lactic acid, (2) proteolysis and (3) fat hydrolysis.

P. roqueforti, like many molds, is favored in its growth by the presence of lactic acid which presumably supplies a satisfactory carbon source and is used for the energy that can be secured. The proteolytic action on milk is evident from the appearance of milk cultures, but can be more definitely shown by determinations of the soluble nitrogen in milk before and after the growth of the organism. The fat hydrolysis during the ripening of Roquefort cheese has been studied in detail by Currie,[67] who has pointed out that it is due chiefly to a water soluble lipase produced by the mold. The hydrolysis results in the accumulation, in both the free and combined forms, of the acids present in milk fat; of these, caproic, caprylic and capric acids and their readily hydrolyzable salts are especially important. This investigator grew the mold on media to which butter fat had been added and found, from the acid number, that more than one-half of the fat had been hydrolyzed. Ethyl acetate, ethyl butyrate, triacetin and tributyrin were also hydrolyzed by a preparation made by triturating air dried mycelium with powdered glass. The solubility of the lipase in water is of special interest in that it explains the fat hydrolysis in parts of the cheese where there is no mold growth.

---

[63] U. S. D. A. B. A. I. Bul. 82. 1906.

[64] U. S. D. A. B. A. I. Bul. 118. 1910.

[65] Jr. Biol. Chem. 15. P. 249. 1913.

[66] See ref. 59.

Protein decomposition in Roquefort cheese. The protein decomposition in a Roquefort cheese is not dependent on the activity of *P. roqueforti* alone since the bacteria and enzymes that are concerned in the proteolysis in hard cheeses generally, are also present. The general nature of the decomposition is illustrated by the following data reported by Currie [68] but secured by Dox.

Nitrogen Distribution in Ripened Roquefort Cheese

Forms of Nitrogen	Per Cent
Insoluble	45.5
Caseoses	10.7
Peptones	8.6
Amino acids	29.1
Ammonia	6.1

These results show the high percentages of insoluble nitrogen and amino acids and the comparatively low percentages of caseoses and peptones that are commonly found in hard cheeses.

The characteristic flavor of Roquefort cheese. Currie [69] has pointed out that the flavor of Roquefort cheese is due largely to the free and combined forms of caproic, caprylic and capric acids liberated from the milk fat. These compounds have a sharp, peppery taste and are responsible for the characteristic effect when the cheese is taken into the mouth.

---

[67] Jr. Agr. Res. 2. P. 1. 1914.

[68] See ref. 67.

[69] See ref. 67.

Since the fats of the milk of various species of animals differ in composition, it would be expected that there might be differences in the suitability of the milk from different species for the manufacture of Roquefort cheese. Currie[70] investigated this from the standpoint of the use of cows' milk and presented the following data, the values for the fat of cows' milk being taken from the work of Browne.

PERCENTAGES OF ACIDS IN THE FATS OF ROQUEFORT CHEESE AND COWS' MILK

Acid	Roquefort Cheese Fat, Per Cent	Cows' Milk Fat Per Cent
Butyric	3.48	5.45
Caproic	4.73	2.09
Caprylic	.58	.49
Capric	3.80	.32
Lauric	5.84	2.57
Myristic	11.36	9.89
Palmitic	28.53	38.61
Stearic	1.91	1.83
Oleic	38.10	32.50
Dioxystearic	....	1.00

He concluded that the differences between the fats from typical Roquefort cheese and from cows' milk are not great enough to necessitate the exclusive use of sheeps' milk in the manufacture of this type of cheese; however, cheese made from sheeps' milk may be expected to have more of a peppery taste than that from cows' milk.

Slime on Roquefort cheese. During the ripening of Roquefort cheese a slime made up of a variety of micro-organisms and commonly including various species of bacteria, yeasts and molds, develops on the surface. Thom, Currie and Matheson,[71] in paraffining experiments, eliminated the slime organisms completely enough to indicate that they have only a secondary function in ripening the cheese. Evans,[72] who found the slime to be made up of characteristic types of micrococci, rod forms and yeasts, concluded that the enzymes from the slime do not appear to be essential to ripening. A normal slime formation is, however, an index to the proper humidity, temperature, etc. in the ripening room.

---

[70] Jr. Agr. Res. 2. P. 429. 1914.

[71] See ref. 59.

[72] See ref. 60.

Molds in blue veined cheeses other than Roquefort. It has often been assumed that *P. roqueforti* is the mold regularly associated with all the blue veined cheeses. Steuart,[73] however, found that in 12 such cheeses of different varieties, this organism was thoroughly dominant in 8, was present in another and was apparently absent in 3. Golding [74] noted that the dominant mold in Wensleydale cheese is not identical with *P. roqueforti.* It seems probable, however, that the changes brought about by the molds in the blue veined cheeses are of the same general nature.

Factors influencing the growth of P. roqueforti. The great variations in the mold growth in the blue veined cheeses suggest that there are various influencing factors. Golding [75] noted that citric and acetic acids had an effect on the growth of different strains of *P. roqueforti.* Low concentrations of acetic acid tended to reduce the digestion of casein in milk while citric acid tended to increase it. In a medium made up of distilled water, various salts, peptone, lactose and agar, acetic acid increased the growth while citric acid tended to inhibit it. Studies carried out by Golding [76] on the salt tolerance of *P. roqueforti* suggested that the salt concentrations usually found in Roquefort cheese may retard but not inhibit the growth of the mold. The composition of the cheese may have such an influence on the growth of *P. roqueforti* that it is an important factor in determining the development of the mold under practical conditions.

BACTERIOLOGY OF CAMEMBERT CHEESE

Camembert cheese is a rennet cheese of the soft type and is typical of this group of cheeses in that the ripening is largely brought about through enzymes elaborated by micro-organisms growing at the surface. The characteristic surface organisms are molds and the enzymes produced by them diffuse in toward the center of the cheese; since the ripening follows these enzymes more or less closely, the outer portion of the cheese ripens first. The action of bacteria in Camembert cheese consists primarily of the development of acid.

S. lactis in Camembert cheese. The formation of acid occurs through the action of *S. lactis.* A culture of the usual type is added to the milk so that this organism is abundant. It encounters very favorable conditions in the young cheese, because of the retention of considerable quantities of water and materials in solution, and continues to develop until large numbers are present. Esten and Mason[77] found that the highest counts usually occurred about the third day, when there was up to several billion organisms per gram. A decrease then began and continued, with fluctuations, until the cheese was ripe. Usually 99 per cent or more of the bacteria in the interior of the cheese were of the lactic acid type.

---

[73] Jr. Dairy Sc. 2. P. 407. 1919.

[74] Mycologia 17. P. 19. 1925.

[75] Jr. Dairy Sc. 9. P. 28. 1926.

[76] Jr. Dairy Sc. 9. P. 236. 1926.

Organisms other than S. lactis in the cheese mass. Various micro-organisms other than *S. lactis* are present in the cheese mass more or less constantly. Esten and Mason[78] found liquefying bacteria, sometimes in considerable numbers, lactobacilli and yeasts. In general, these organisms are present because of their ability to develop in an acid medium; they apparently play no necessary part in the making or ripening processes.

Typical mold of Camembert cheese. The characteristic mold of Camembert cheese is *Penicillium camemberti;*[79] a white variety occurs and has been designated *P. camemberti var. rogeri.* This species seems to be rather strictly limited in its growth to Camembert cheese. Thom[80] reported that, ''Persistent search has failed to find a single colony in America whose presence can be attributed to anything but Camembert cheese imported from Europe.''

In the United States the mold is commonly inoculated on the surface of the cheese soon after making. It can be grown on water crackers[81] that have been sterilized in a container and then moistened with sterile water to which mold from a stock culture has been added; in some instances a 5 or 10 per cent aqueous solution of lactic or tartaric acid is used instead of the water. The cheese can be inoculated by sprinkling with a water suspension of the mold or this may be sprayed on. French makers commonly believe it is unnecessary to inoculate after the mold is once established in the plant because the organism is added to the cheese from the air and equipment.

P. camemberti is commonly evident on the cheese several days after making and then spreads rapidly over the surface. Later, when spore formation begins, the mold may be still more conspicuous.

---

[77] Conn. (Storrs) Agr. Expt. Sta. Bul. 83. 1915.

[78] See ref. 77.

[79] See ref. 64.

[80] See ref. 63.

[81] U. S. D. A. Bul. 1171. 1923.

Growth of O. lactis. *O. lactis* commonly develops along with *P. camemberti* on the surface of the cheese and, in some instances, even precedes it. The nutrients, moisture, etc., available are very satisfactory for the growth of this species and it would commonly overgrow the other organisms if it were not for the inhibiting effect of the salt. Camembert cheese usually contains from 2.5 to 3.0 per cent salt and this tends to hold the development of *O. lactis* down to a desired point.

Slime formation. Following the conspicuous growth of molds on the surface of the cheese there normally occurs the formation of a slime which is made up largely of bacteria and yeasts. It may have a red or yellow color and is then usually taken as an indication of a good quality cheese. In general, the slime tends to cover up the mold growth.

The numbers of bacteria and yeasts in the slime have been investigated by Esten and Mason;[82] a portion of their data are as follows:

BACTERIA AND YEASTS IN CAMEMBERT CHEESE SLIME

Origin of Cheese	Bacteria per Gram
Made at Storrs....	19,375,000,000
Made at Storrs....	7,375,000,000
Made at Storrs....	12,166,700,000
Imported.........	57,567,000,000
Imported.........	28,050,000,000
Imported.........	259,333,300,000
	Yeasts per Gram
Made at Storrs....	852,500
Made at Storrs....	1,062,000
Made at Storrs....	3,576,000
Imported.........	652,500
Imported.........	35,790,000

From these results it is evident that enormous numbers of bacteria and yeasts are present. In all probability these organisms do not play a necessary part in the ripening process; however, they tend to limit mold growth and may form a coating which partially excludes the air and thus aids in controlling the development of off flavors.

The conditions provided at the surface of Camembert cheese during the ripening are very favorable for the development of many species of micro-organisms. The humidity of the ripening room is normally high, nutrients are available from the cheese mass and the lactic acid supplied through the activity of *S. lactis* favors certain yeasts and molds. It is probable also that associative action among the various species makes possible an extensive development of certain of them.

---

[82] See ref. 77.

Importance of molds in Camembert cheese. In distinction to the blue veined cheeses, the necessary mold growth in the Camembert type is limited to the surface. The principal action of *P. camemberti* in the ripening of the cheese is to bring about proteolysis. The proteolyzing action of this mold can be shown by inoculating sterile curd, when the decomposition products formed are found to be essentially the same as those in Camembert cheese. The enzyme produced diffuses from the surface growth and, through its decomposition of the casein or casein derivatives, brings about pronounced changes which influence the texture and the flavor. At certain stages the outer portion of the cheese is ripe while the inner portion is still green. *P. camemberti* is also active in reducing the acidity in the cheese; this occurs partly through the use of the acid for the energy

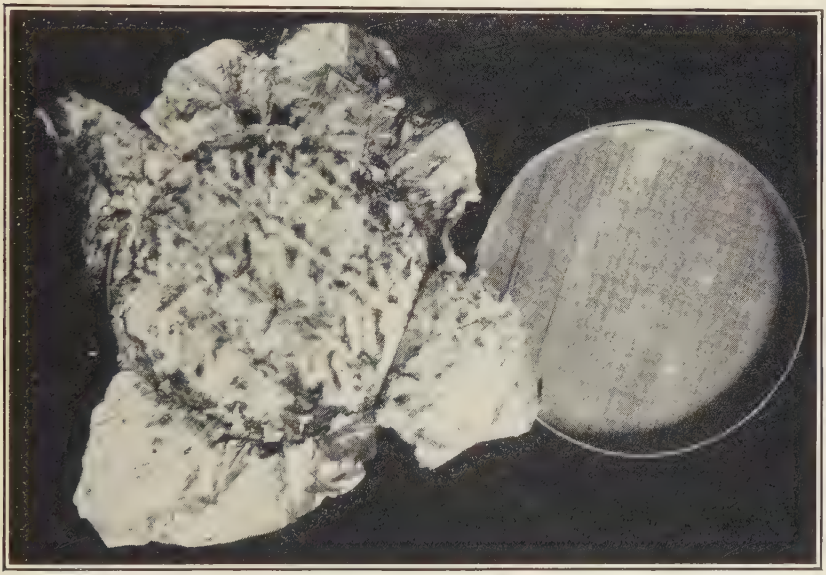


FIG. 46.—Open package of Camembert cheese (domestic) showing normal surface growth.

that can be secured and partly through neutralization by the compounds formed.

O. lactis is important in connection with the flavor and aroma development. If this organism is absent or limited in its growth, the ripening may appear to progress in a normal manner but the cheese lacks flavor. An over development may also occur and the layer of growth at the surface of the cheese is then thick and wrinkled and does not adhere properly; under these conditions the cheese ripens abnormally because of the failure of *P. camemberti* to grow as it should.

Proteolysis in Camembert cheese. The principal agent in the protein decomposition in Camembert cheese is the enzyme produced by *P. camemberti.* The enzymes pepsin and galactase, which are important in the ripening of some of the hard cheeses, may be active but apparently are not necessary for normal ripening. Bosworth[83] has pointed out that the only function of the rennet is to coagulate the milk. This investigator found that the water soluble nitrogen does not increase appreciably until the cheese is 10 days old but that finally the proteolysis is extensive, over 80 per cent of the nitrogen being water soluble after four weeks. There is considerable amid nitrogen formed and, toward the end of the ripening, some ammonia.

Numbers of bacteria in ripened Camembert cheese. Although the maximum numbers of bacteria in Camembert cheese are present soon after making and a decrease then begins, there may be large numbers present in the cheese mass when ripening is complete. The following results, taken from the data of Esten and Mason,[84] illustrate this.

BACTERIA IN RIPENED CAMEMBERT CHEESE

Origin of Cheese	Bacteria per Gram
Made at Storrs....	858,000,000
Made at Storrs....	365,500,000
Made at Storrs....	2,300,000,000
Made at Storrs....	45,417,000
Imported.........	10,968,000
Imported.........	6,250,000
Imported.........	254,000,000
Imported.........	442,100,000

[83] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 5. 1907.

[84] See ref. 77.

The great variation in these counts is of interest. The decrease in the numbers from the maximum is due to the more or less unfavorable conditions for the development of bacteria and it would be expected that variations would occur from one cheese to another; the age of the cheese is undoubtedly also a factor.

Manufacture of Camembert cheese from pasteurized milk. Hochstrasser and Price[85] reported that milk pasteurized at 62.8° C. (145° F.) for 30 minutes produced Camembert cheese of a better average quality and greater uniformity than raw milk. The improvement was greatest when the original milk was of an inferior grade. The quality of the pasteurized milk cheese improved as the quality of the original milk improved. These investigators noted that pasteurization had practically no influence on the yield of ripened Camembert cheese.

BACTERIOLOGY OF COTTAGE CHEESE

Cottage cheese belongs to the group of cheeses with which the curdling of the milk is primarily due to acid development. The bacterial changes yielding the desired flavor and aroma very largely occur during the coagulation of the milk so that the cheese is ready to eat as soon as it is made. Raw milk may be allowed to develop acid spontaneously but the usual practice under commercial conditions is to pasteurize the milk and then add a good culture of the type used in the butter industry. The character and flavor of the curd may be modified by the temperature to which it is heated and various other procedures, so that there is a wide variation in cottage cheese as it appears on the market.

Sources of the flavor. The desirable flavor of cottage cheese is largely the result of the growth of the organisms contained in butter cultures; these are quite regularly present in milk so that they may be active in milk allowed to sour spontaneously, but under these conditions other types of organisms very often have an undesirable influence on the flavor. The changes brought about by the culture organisms are the same as when these organisms are developing in milk to be made into culture, and cottage cheese may be looked upon as culture from which a considerable portion of the water and materials in solution have been removed. The quality of the culture, accordingly, has a pronounced influenced on the flavor and aroma of the cheese.

---

[85] Jr. Dairy Sc. 10. P. 448. 1927.

Importance of pasteurizing the milk. In the manufacture of good flavored cottage cheese efficient pasteurization of the milk is very important. Without it, the flavor and aroma are practically certain to be influenced by various organisms present in the milk that find an opportunity for growth during the period when the milk is being held at a temperature favorable for acid development. Gas forming organisms, especially those of the Escherichia-Aerobacter group, may become active so that the curd is filled with gas holes. Hammer[86] reported gas formation in a vat of milk set for cottage cheese that was so pronounced the gassy curd was raised up over the edge of the vat. The gas forming organisms ordinarily produce a very undesirable flavor and aroma.

Deterioration of cottage cheese. The acid present in cottage cheese inhibits the development of many species of micro-organisms, but others, especially the molds and yeasts, tolerate this acid and their growth may even be accelerated by it; the considerable percentage of water in the product also tends to favor them. Accordingly, cottage cheese has poor keeping qualities at the temperatures encountered in retail establishments.

Molds appear at the surface of the cheese and in air spaces along the wall of the container. There may be many centers of growth, if careless methods have been used, or only an occasional one suggestive of air contamination. The color and general type of growth vary greatly because of the variety of species involved. Molds not only spoil the appearance of the cheese but also influence the flavor; some of them have pronounced proteolyzing powers and, if given time, digest considerable portions of the curd.

Various yeasts develop where there is an abundant air supply while some species are capable of growth through the cheese mass. Considerable surface growth of the types forming white colonies may occur without it being especially noticeable, because of the lack of contrast. Colonies of the pink yeasts are very conspicuous; in general, these organisms have little effect on the flavor and aroma of the cheese, even when growth is abundant. A typical yeasty flavor and aroma are usually due to the lactose fermenting species; this condition is especially common in cheese made from raw milk.

Deterioration of cottage cheese, through the growth of molds and yeasts, may occur even when the product is made from pasteurized milk, due to contamination subsequent to the heating. These organisms gain entrance to the milk, or the cheese, from the utensils and equipment, from the air and various other sources; without proper care the drain cloths may be a very serious source of contamination. Organisms getting into the milk have a good chance for growth during the period the milk is allowed to stand at a temperature favorable for acid development.

---

[86] Ia. Agr. Expt. Sta. Res. Bul. 27. 1916.

Warren[87] studied a sharply acid and bitter flavor in pimento cheese of the soft, unripened Neufchatel type and found that it was due to an organism resembling *C. butyricum.* Nine samples of the abnormal cheese contained large numbers of this organism and the characteristic defect was produced experimentally by the use of a pure culture of it. Spores of the organism which had been shocked by heating to 85° C. (185° F.) for 15 minutes gave a more active fermentation than the unheated spores. The associative action of *S. lactis* and the anaerobe proved more effective than the anaerobe alone in developing the defect.

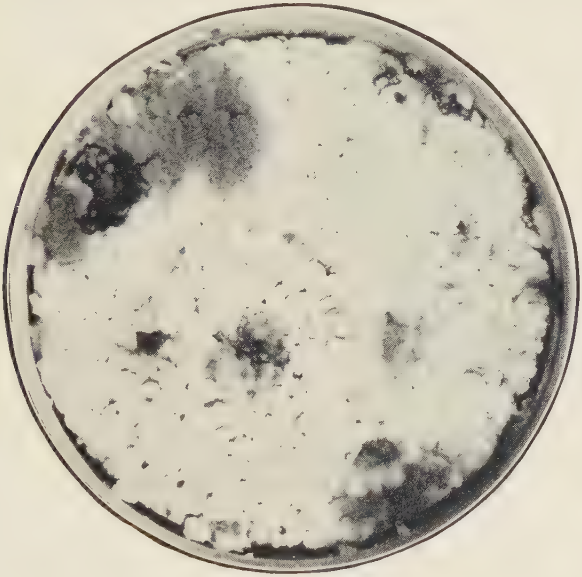


Fig. 47.—Mold growth on cottage cheese.

Addition of cream to cottage cheese. Many manufacturers add cream to cottage cheese and, in this way, get a product which is especially pleasing to many consumers. In general, however, the addition of cream decreases the keeping quality of the product.

---

[87] Jr. Dairy Sc. 9. P. 351. 1926.

Preservation of cottage cheese. The principal method of controlling the deterioration of cottage cheese is through the use of low temperatures. It can be held at 0° C. (32° F.) for a considerable period without showing changes, while at 10° C. (50° F.), or higher, it keeps for a much shorter time. Careful methods of making and packing aid in controlling the entrance of molds and yeasts and thus have an effect on the keeping quality.

Cottage cheese is sometimes packed in large containers and held for considerable periods in a frozen condition; on removal from storage it is ordinarily mixed with cream. When frozen the cheese often keeps very well but off flavors, such as high acid, stale, bitter, metallic, etc., may develop, presumably due to the growth of organisms before the cheese is frozen or to chemical action. Ellenberger[88] noted that the keeping quality of cottage cheese after removal from storage was nearly although not quite as good as that of fresh cheese.

[88] Vt. Agr. Expt. Sta. Bul. 213. 1919.

CHAPTER XVI

TESTS FOR THE QUALITY OF MILK AND CREAM

Various tests, other than the enumeration of the contained organisms, have been devised for the determination of the quality of milk and cream. Some of these depend, in a general way, on the numbers of organisms present but are intended primarily for use under plant conditions and give results quickly and with comparatively little work or apparatus; commonly the technique is easily carried out so that it can be taught to persons without training along bacteriological lines. Other tests are expected to detect the presence of organisms that are undesirable in certain manufacturing processes, such as cheesemaking, or that indicate the contamination of milk from objectionable sources, and do not involve the total numbers of organisms in any way.

Most of the tests for the quality of milk and cream fall into three groups as follows: (1) those involving the detection of the products of the growth of micro-organisms, (2) those involving the rate at which changes are produced by the contained micro-organisms and (3) those involving the presence of certain types of micro-organisms.

TESTS INVOLVING THE DETECTION OF THE PRODUCTS OF THE GROWTH OF MICRO-ORGANISMS

Under the usual conditions any extended growth of micro-organisms in milk or cream results in the formation of products that give an abnormal flavor and aroma and, when extensive enough, a definite increase in acid. The presence of these products constitutes the basis of some of the tests that are used with milk and cream.

Examination for Flavor and Aroma

Milk and cream are often examined for flavor and aroma. The odor of a can of milk or cream when the cover is first raised is especially valuable in indicating a slight abnormality. An acid flavor and aroma is the condition most often encountered but flavors and aromas of a variety of types are frequent. The senses of taste and smell are much more acute in some persons than in others and certain individuals find it impossible to develop the ability to accurately detect slight abnormalities in flavor and aroma.

The off flavors and aromas due to micro-organisms should be carefully distinguished from those due to other causes so that the producer can be advised as to the proper methods to employ in the correction of the difficulty.

Titration for an Increased Acidity

Milk and cream are often titrated for the purpose of detecting an increased acidity. This necessitates the establishment of a standard for the sweet products and the maximum allowed is usually 0.2 per cent. There is considerable variation in the initial acidity of milk, depending in part on the composition, and this results in errors with two types of milk, (1) milk with a very low bacterial count but high in solids so that the initial acidity is over 0.2 per cent and (2) milk with such a low initial acidity that, even after a definite increase, the acidity is still under 0.2 per cent. There is less danger of serious errors with the acid test when the milk from a herd is being dealt with than when the milk comes from one animal. Cream which shows an acidity of 0.2 per cent has definitely developed acid because its initial acidity is lower than that of the milk from which it came.

The acid test has proved very valuable in the grading of cream intended for buttermaking. The off flavors and aromas due to micro-organisms are ordinarily the most serious and these are not commonly conspicuous in cream having an acidity of 0.2 per cent or less. The rapid grading of cream on any acid basis that is selected may be carried out by mixing cream and standard alkali in definite proportions and then noting whether the reaction is acid or alkaline. Milk and cream with a slight developed acidity cannot be used for certain purposes and the acid test is an aid in the detection of these products. From the bacteriological standpoint, however, the test is very crude since considerable bacterial development is commonly required before an appreciable increase in acidity results and numbers of organisms running into the millions per cubic centimeter often go undetected. One of the important advantages of the acid tests is the convenience and rapidity with which they can be used.

ALCOHOL TEST

The alcohol test consists of mixing, in a test tube, equal parts of milk and ethyl alcohol of a definite percentage and noting whether or not coagulation occurs; with normal milk, the mixture breaks clear from the wall of the tube while, with milk showing certain abnormalities, the wall is clouded with fine particles of curd and the mixture shows a precipitation of the milk constituents. The alcohol that has been employed has usually varied in strength from 68 to 75 per cent by volume. It can be prepared by diluting high grade alcohol with distilled water. The diluted alcohol should be tested for acid, as acetic acid is sometimes present and may influence the test; alcohol containing acid should be distilled in the presence of sodium hydroxide. The test is usually carried out at room temperature, using two cubic centimeters of milk.

Conditions causing coagulation. From the data available it is evident that the products of the growth of micro-organisms may be responsible for the coagulation in the alcohol test and also that certain other factors may be involved.

One of the causes of a positive test is the presence of developed acid in milk. Dahlberg and Garner,[1] however, found no direct relationship between the coagulation of milk with alcohol and its titratable acidity, although milk high in acidity gave coagulation in the majority of the cases. The development of a bacterial rennin in milk may be a factor in the test; Sommer and Binney[2] noted that the coagulating enzyme produced by 856,000 rennin forming organisms per cc. was sufficient to give a positive result. The data of Ayers and Johnson[3] are important in showing the influence of the products of bacterial growth on the test. These investigators found no definite relationship between the numbers of bacteria in milk and the coagulation with alcohol. Of 177 samples of raw milk studied, 19 were positive with 68 per cent alcohol and, of these, 8 (42.1%) contained less than 500,000 bacteria per cc. and 11 (57.9%) contained more than 500,000; among the samples that were negative, a considerable number contained over 500,000 bacteria per cc., the maximum being 20,200,000.

Colostrum milk and milk from cows advanced in lactation are usually positive with the alcohol test and the same is true of some lots of milk from abnormal udders. This suggests that certain abnormalities in the composition of milk result in coagulation when the milk is mixed with the alcohol. If milk that coagulates with alcohol is mixed with normal milk the mixture may not coagulate; Ayers and Johnson[4] found that a mixture of colostrum and normal milk would not give a positive test unless a very large percentage of the milk was colostrum.

---

[1] U. S. D. A. Bul. 944. 1921.

[2] Jr. Dairy Sc. 6. P. 176. 1923.

[3] U. S. D. A. Bul. 202. 1915.

Sommer and Binney[5] investigated the influence of the salt balance of milk on the alcohol test and noted that a slight increase in the magnesium or calcium content gives a positive result while an increase in potassium, sodium, chloride, citrate or phosphate does not. The effect of the magnesium and calcium is counteracted by the citrates and phosphates so that a positive test depends mainly on the relative amounts of the four constituents. These investigators found that a high calcium content in a ration fed to cows may cause a positive alcohol test in the milk.

Weimar[6] reported instances in which a positive alcohol test was due to the feeding of silage of a poor quality. A study of samples of milk from individual animals, only a part of which were positive, indicated that the bacterial content and acidity were of no importance and suggested the influence of the silage since the cows giving milk that was negative had refused the silage. Experimental feeding with three animals showed that the effect of alcohol on the milk could be changed by varying the feed.

Modifications of the alcohol test. Methods of titrating with alcohol have been devised, the end point being considered the first appearance of a coagulation in the milk. In general, these do not have an advantage over the method of mixing equal volumes of alcohol and milk.

Chalmers[7] has modified the alcohol test by using a preliminary incubation period of at least nine hours at 37° C. (98.6° F.) with the milk and then adding 83 per cent (by volume) neutral alcohol containing 0.02 per cent methyl red. The incubation permits the development of the contained organisms under standard conditions before the examination for the products of their growth is made, while the indicator gives information as to the acidity present. The cooling of the milk before the reagent is added appears to be important from the standpoint of the color obtained with the indicator.

Uses of the alcohol test. The alcohol test has been suggested for use with milk intended for condensing and drying. Its detection of unusual salt balances in addition to certain other abnormalities may prove very important.

---

[4] See ref. 3.

[5] See ref. 2.

[6] Jr. Dairy Sc. 6. P. 95. 1923.

[7] Jr. Bact. 15. P. 93. 1928.

TESTS INVOLVING THE RATE AT WHICH CHANGES ARE PRODUCED BY THE CONTAINED MICRO-ORGANISMS

The rate at which a change is produced by the micro-organisms present in milk or cream affords a basis for a number of tests used to arrive at the bacteriological condition of these products.

Keeping Quality Tests

A simple keeping quality test may be made on lots of milk or cream by holding small amounts under more or less definite conditions and observing, from time to time, for the appearance of changes. The portions may be held in test tubes or small bottles that have been sterilized or thoroughly steamed and should be transferred under conditions which prevent contamination. A constant temperature incubator is preferable for holding but is not necessary and the samples in a series may be held at any temperature reasonably suitable for the growth of the organisms. A determination of the time of coagulation seems to be the most convenient basis for comparison; although the per cent of acid at which this occurs varies with different lots of milk, it seems to be more satisfactory than the development of an acid flavor or aroma because of the difficulty of accurately judging the presence of these.

The character of the curd formed may give information of importance from the standpoint of the types of organisms present in the milk. Gas producing organisms, rapid casein decomposers and types producing objectionable odors may often be detected by an examination of the curdled material. Such information is of special significance with milk to be made into certain types of cheese.

Keeping Quality in the Presence of an Indicator

Baker and Van Slyke[8] have proposed a method of determining the keeping quality of milk in the presence of brom-cresol purple, which makes possible the recognition of slight changes in acidity. Three cubic centimeters of milk and one drop of a saturated aqueous solution of the indicator are mixed in a tube, with precautions to prevent contamination, and incubated at a definite temperature, usually 18° to 20° C. (64.4° to 68° F.). The milk is examined for changes in color at various intervals. As the acid increases the color varies from the grayish-blue in normal milk to a final clear yellow. Changes involving digestion, gas formation, etc. are also evident after prolonged incubation.

---

[8] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 72. 1919.

The use of brom-cresol purple in milk was found by Baker and Van Slyke[9] to make possible the preliminary detection of abnormal milks. The color is made lighter than normal by acids, acid salts, formaldehyde and also by heating above the usual temperature of pasteurization, while it becomes darker in the case of milk from diseased udders, watered milk, skim milk and milk containing alkali or alkaline salts.

Changes Produced in *p*H of Bouillon by Organisms in Added Milk

Cooledge and Wyant[10] suggested a method of determining the keeping quality of milk by a colorimetric *p*H test, using 0.1 cc. of the milk in bouillon containing an indicator. The general procedure is as follows:[11] One bouillon cube, one per cent peptone and one-half per cent sodium chloride are added to each liter of water and, after heating and filtering, 80 cc. of a 0.04 per cent aqueous solution of brom-thymol blue are added. The broth is cooled to 20° C. (68° F.) and the *p*H adjusted to 6.8. It is then placed in 100 or 200 cc. flasks and sterilized. When a flask of the bouillon is to be used, 10 cc. are transferred to a 1.8 by 16 cm. sterile test tube with a sterile pipette and compared with a standard having a *p*H of 7.0; if incorrect, the broth in the flask is adjusted to *p*H 7.0. The broth is transferred to sterile 1.8 by 16 cm. test tubes, 10 cc. in each. One-tenth cc. of each milk sample is then added to a tube of the broth, using a 9 cc. blank, and the tubes incubated at 37° C. (98.6° F.). Hourly observations are made to detect the first color change due to the development of acidity. As soon as pronounced changes are noticed, hourly readings are made by comparing the tubes with standards of known *p*H values. The broth must be cooled to 20° C. before readings are made.

Cooledge and Wyant[12] have pointed out that the method makes it possible to detect the poorer samples of milk at the end of one hour, while the best samples can be given their proper grade at the end of eight hours; the poorer the keeping quality of the milk, the sooner the results are obtainable. These investigators have also suggested a method of scoring milk on the basis of the time required for the development of a definite *p*H value. Cooledge[13] found that the method checked very well with the keeping quality of milk. He also worked out a procedure for grading milk and paying a bonus to farmers furnishing milk with a good keeping quality, as determined by the *p*H test. Cooledge and Goodwin[14] reported that the test had proved satisfactory for judging a milk contest. Cooledge[15] studied the test as a means of locating faulty methods in city milk plants and found it very useful; it appeared to be suitable for controlling the pasteurization process at either small or large plants.

---

[9] N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 71. 1919.

[10] Jr. Dairy Sc. 3. P. 156. 1920.

[11] Mich. Agr. Expt. Sta. Spec. Bul. 124. 1923.

[12] See ref. 10.

METHYLENE BLUE REDUCTION TEST

The methylene blue reduction test, or the reductase test, involves the determination of the time required for the disappearance of the color when a methylene blue solution is added to milk under standard conditions.[16]

The apparatus required is either thoroughly boiled just before use or sterilized. Boiling is entirely satisfactory since the few organisms surviving this treatment are of little importance in causing the reduction. The methylene blue solution is commonly made up by dissolving a standard tablet in 200 cc. of water that has been boiled and cooled, but may also be prepared by dissolving methylene blue in water; the proportion that has been recommended with methylene blue is one part to 20,000 parts of water but this gives a higher color than is secured with the tablets. It has been suggested that the methylene blue solution should not be used for more than a few days because of the deterioration that occurs; however, this is not in agreement with some of the observations reported.

One cc. of the methylene blue solution is added to 10 cc. of milk in a test tube and thoroughly distributed. The temperature is quickly brought to 37° C. (98.6° F.) in a water bath and the tube incubated at this temperature. The time required for the disappearance of the color is then noted. The frequency of the observations is determined by the number of groups into which it is desired to divide the samples. The following grouping has apparently been used more frequently than any other.

---

[13] Mich. Agr. Expt. Sta. Tech. Bul. 52. 1921.

[14] Mich. Agr. Expt. Sta. Spec. Bul. 112. 1921.

[15] See ref. 11.

[16] Standard Methods of Milk Analysis. 5th ed. P. 25. 1927.

Class 1. Good milk, not decolorized in five and one-half hours; develops, as a rule, less than 500,000 colonies per cc. on agar plates.

Class 2. Milk of fair quality, decolorized between two and five and one-half hours; develops, as a rule, between 500,000 and 4,000,000 colonies per cc. on agar plates.

Class 3. Bad milk, decolorized between 20 minutes and two hours; develops, as a rule, between 4,000,000 and 20,000,000 colonies per cc. on agar plates.

Class 4. Very bad milk, decolorized in 20 minutes or less; develops, as a rule, over 20,000,000 colonies per cc. on agar plates.

Influence of various factors on the methylene blue test. The influence of various factors on the methylene blue test has been investigated by Hastings, Davenport and Wright.[17] The reduction time was prolonged as the concentration of the dye was increased. The age of the methylene blue solution had little if any effect on the reduction time, solutions two years old giving essentially the same results as fresh solutions. In the investigation of the influence of temperature, 28° C. (82.4° F.), 33° C. (91.4° F.) and 38° C. (100.4° F.) were used; with the majority of the samples the highest temperature gave the most rapid reduction but, with certain of them, the reduction time at 28° C. was shorter than at 38° C. The bacteria common in milk show great variations in their optimum temperatures and, since certain of these are considerably below 38° C., it would be expected that some samples might reduce more rapidly at lower temperatures than at 38° C.

The cause of the reduction. Hastings, Davenport and Wright[18] found that temperatures which are not supposed to affect enzymes influenced the reduction of milk and that such a weak antiseptic as boric acid in a concentration of one part to 2,500 parts of milk approximately doubled the reduction time; from these results they concluded that the reduction is very intimately connected with the vital processes of the cell rather than with any extracellular byproducts.

Variations in reducing power of different organisms. Different species of organisms vary a great deal in their reducing power. *S.*

---

[17] Jr. Dairy Sc. 5. P. 438. 1922.

[18] See ref. 17.

lactis reduces very rapidly and is undoubtedly one of the organisms mainly responsible for the very rapid reduction of milk containing large numbers of bacteria. The variations in the reducing powers of different species indicate that the correlation between the time of reduction and the number of organisms originally present can not be an exact one; moreover, Hastings, Davenport and Wright[19] secured results which make it seem that the reduction test reflects only in a very general way the keeping quality of a sample of milk.

Value of the methylene blue test. Hastings[20] has presented data indicating that the methylene blue test measures, with a high degree of accuracy, the bacterial content of milk samples prepared by the addition of different amounts of the same inoculum. Hastings and Davenport[21] found this test preferable to either the keeping quality test in the presence of brom-cresol purple or the test in which the change in the *p*H in bouillon is observed, because of its greater sensitiveness to biological differences in milk.

Troy[22] investigated the correlation between the methylene blue test and the direct microscopic count in the grading of milk, a definite reduction time and a definite direct count being chosen for each of three grades. Out of 1,947 samples, there were 266 with which the methods did not agree as to the grade in which the milk should be placed; of these, 182 were placed in the lower grade by the direct microscopic count while only 84 were placed in the lower grade by the reduction test. In the instances in which the two methods did not agree, the milk was usually placed in adjoining grades but, in a few cases, one method put a sample in the highest grade while the other put it in the lowest grade.

Hiscox and Starling[23] found that the usual division of milk into four grades with the methylene blue test does not satisfy the requirements of the English system of grading, since it does not discriminate between milk of an average quality and that of a superfine quality. They concluded that, if a reduction time of five and one-half hours is retained as a minimum for milk of the highest class, there is no certainty that milk of a very poor quality may not be included while, if it is increased, there is danger that milk of a very good quality may be excluded.

In a comparison of the methylene blue test and the agar plate count Ellenberger, Bond, Robertson and Moody[24] noted less variation between check or duplicate tests with the former than with the latter; they also found that the methylene blue test correlated much more closely with the keeping quality of the milk than did the agar plate count.

---

[19] See ref. 17.

[20] Jr. Dairy Sc. 2. P. 293. 1919.

[21] Jr. Dairy Sc. 3. P. 353. 1920.

[22] Jr. Dairy Sc. 8. P. 282. 1925.

[23] Jr. Hyg. 24. P. 164. 1925.

[24] Vt. Agr. Expt. Sta. Bul. 264. 1927.

The methylene blue test is one of the very useful methods of determining the general bacteriological condition of milk. It has been widely employed in Europe and is becoming of increasing importance in the United States. By observing a few simple precautions the procedure can be carried out by a person without bacteriological training and the expense for the apparatus is small. The test offers possibilities in connection with the control of the quality of the milk delivered to cheese factories,[25] especially since it can be combined with the fermentation test and information secured on the presence of objectionable types of organisms, as well as on the time required for reduction. It can readily be used with sweet cream purchased for various purposes and the extremely rapid reduction secured with samples containing excessive numbers of organisms shows the patron the objectionable condition in a striking manner. The test has also been suggested for use in determining the bacteriological condition of ice cream.

It should be definitely recognized that the methylene blue test does not determine the number of organisms but simply measures one type of their activity and that, accordingly, no close agreement can be expected between bacterial counts and the results obtained with it. The great variation in the reducing power of different species is a complicating factor of importance. In an instance observed at the Iowa Agricultural Experiment Station, in which the reduction time with a sample of milk was much shorter than would be expected from the bacterial count, an organism was isolated that, in pure culture, reduced methylene blue very quickly.

Grimes, Barrett and Reilly[26] have pointed out that, the longer the reduction time, the greater the variation between duplicate or triplicate determinations. This suggests that very small differences in the organisms present, such as are to be expected with two or more portions of milk measured from the same lot, may influence the rate of reduction of methylene blue through the variations that occur in the growth over an extended period.

[25] Wis. Agr. Expt. Sta. Cir. 204. 1926.

[26] Scien. Proc. Roy. Dublin Soc. 18. n. s. P. 437. 1927.

TESTS INVOLVING THE PRESENCE OF CERTAIN TYPES OF MICRO-ORGANISMS

The presence of a certain type of micro-organism in milk or cream sometimes permits of more or less definite conclusions with reference to these products and a number of tests involving this principle have been proposed. The organisms considered in these tests may be objectionable because of an undesirable change brought about during some manufacturing process, such as the formation of gas in cheese, or they may be associated with contamination from a definite source.

Wisconsin Curd Test

The Wisconsin curd test is used in the detection of milk that is unsatisfactory for cheesemaking; it shows especially the presence of objectionable gas forming organisms but may also give information with reference to other undesirable types.

Method of making the test. The samples of milk to be examined are collected in sterile pint jars or wide mouth bottles, the containers being filled almost full. By means of a water bath, the samples are adjusted to a temperature of about 32.2° C. (90° F.) and 10 drops of rennet extract mixed with each. As soon as a firm curd is formed, it is cut into small pieces with a knife that has been sterilized, or at least thoroughly boiled, and then, as the whey collects, this is poured off. When a small pat of curd quite free from whey is secured, the container is put at a temperature of about 37° C. (98.6° F.). After 10 or 12 hours the curd is examined. If it is solid throughout, shows no sliminess at the surface and has an agreeable odor, the milk was probably free from organisms that would be objectionable in cheesemaking, while gas holes in the curd, sliminess, objectionable odors, etc., show the presence of organisms that can be expected to cause undesirable conditions in cheese; in some instances the curd is so full of gas holes that it may be spongy. Milk with a very high bacterial count may give a desirable type of curd and another lot with a low count may give a very undesirable curd so that the test is of no value in comparing samples of milk from the standpoint of their keeping qualities.

Value of the test. The use of the Wisconsin curd test on the milk from individual patrons makes it possible for the cheesemaker to pick out milk that is objectionable for cheese manufacture. This is very important since one lot of seriously contaminated milk may be sufficient to cause an undesirable condition in the entire day's make.

The results of the test are not available before the cheesemaking is begun but there is ordinarily a general similarity in the milk from a farm from day to day so that the cheesemaker can detect the farms where an improvement in the methods of milk production is necessary.

ANAEROBIC SPORE TEST

Some of the spore forming anaerobic organisms produce a "stormy" fermentation in milk that is more or less characteristic and certain investigators have attempted to establish the significance of the appearance of this change in milk held under conditions favorable for it. Weinzirl and Veldee[27] called attention to the possibility of testing milk for contamination with manure in this way. The procedure involved heating milk in definite amounts, such as 5, 10 and 15 cc., to 80° C. (176° F.) for 10 minutes, covering with sterile paraffin to a depth of about one-eighth inch and then cooling and incubating at 37° C. (98.6° F.). If anaerobes are present, gas will be formed and will force up the paraffin plug. These investigators considered that the determination of the amount of milk required to give a gassy fermentation, under the conditions used, made it possible to secure information on the extent of contamination with manure. Later, Weinzirl[28] used the following procedure. One-half to one cc. of melted paraffin is placed in a 1.5 cm. test tube which is plugged with cotton and sterilized, either with dry or moist heat. By means of a sterile pipette, 5 cc. of the milk to be tested is placed in each of 5 tubes containing paraffin and the tubes then heated in an Arnold sterilizer to 80° C. for 10 or 15 minutes. The melted paraffin rises to the surface where it hardens when the temperature is lowered. The tubes are incubated for three days at 37° C. If 2 tubes out of the 5 form gas the milk is considered to show excessive pollution.

In the studies carried out by Ayers and Clemmer,[29] ten tubes of milk, each containing 20 cc., were used for a sample and there was a fairly definite relationship between the results and the conditions of production, but it was noted that no reliance could be placed on one test with a sample and that a series of tests on a number of samples was necessary for the results to have any significance.

Hudson and Tanner[30] compared the anaerobic spore test with the

---

[27] Am. Jr. Pub. Health. 5. P. 862. 1915.

[28] Am. Jr. Pub. Health. 11. P. 149. 1921.

[29] U. S. D. A. Bul. 940. 1921.

[30] Jr. Dairy Sc. 5. P. 377. 1922.

Frost plate method and the sediment test on over 100 samples of milk. They concluded that the procedure, as used, can not be accepted as a method of accurately determining manurial pollution in milk; no correlation was found between the results of the test and the number of bacteria or the amount of dirt.

The anaerobe which is of the most importance in causing gas in heated milk held in deep layers seems to be *Clostridium sporogenes* but there are undoubtedly other species also involved. The results of Ayers and Clemmer[31] indicate that the majority of the spores of *C. sporogenes* gain entrance to milk from cow manure. It should be recognized, however, that there are other sources from which spores of anaerobes may get into milk.

TESTS FOR ESCHERICHIA-AEROBACTER ORGANISMS

The importance of the Escherichia-Aerobacter organisms in water as an indication of pollution has led to attempts to use them in determining the quality of milk. Because of the opportunities for the contamination of milk with these organisms, even when reasonably careful methods of production are used, no significance can be attached to their mere presence but determinations of the number per cubic centimeter may permit of conclusions under certain conditions.

The Escherichia-Aerobacter organisms can be determined by inoculating various quantities of milk into a suitable medium and then observing for gas formation; Kessler and Swenarton[32] found that gentian violet peptone bile was very satisfactory for this purpose. The organisms can also be determined by plating on any one of a number of media[33] with which they give more or less characteristic colonies. The numbers of Escherichia-Aerobacter organisms per cubic centimeter is sometimes referred to as the colon count.

There is no relationship between the number of Escherichia-Aerobacter organisms in milk and the total bacterial count and none would be expected because of the variety of sources from which organisms get into milk and the variations in the types from each source. A colon count, accordingly, can not replace the total bacterial count. The New Hampshire State Board of Health[34] used the colon count along with the total count in detecting objectionable conditions that were not evident from the total count alone; it was found that milk may have a comparatively low total count, due to adequate cooling, and yet show excessive numbers of Escherichia-Aerobacter organisms as a result of contamination.

---

[31] See ref. 29.

[32] Jr. Bact. 14. P. 47. 1927.

[33] Ia. Eng. Expt. Sta. Bul. 62. 1921.

[34] N. H. State Bd. Health. 29th Rpt. P. 152. 1926.

It should be noted that Escherichia-Aerobacter organisms get into milk from various sources so that large numbers of them do not necessarily mean extensive fecal contamination. Ayers and Clemmer[35] concluded that, in fresh milk, the colon count does not indicate the extent of direct manurial contamination but does indicate the general conditions of cleanliness under which the milk was produced and that, in milk of unknown history, no significance can be attached to the count other than that high counts usually indicate high holding temperatures or very abnormal conditions of production.

The presence of Escherichia-Aerobacter organisms in pasteurized milk has sometimes been assumed to indicate inefficient pasteurization or contamination following heating, but various investigators have found organisms of this group which resisted satisfactory pasteurization exposures. Swenarton[36] proposed a standard for pasteurized milk which permits some of the tests made with standard 0.1 cc. portions of the milk to contain Escherichia-Aerobacter organisms.

Tests for Streptococci

Because of the importance of streptococci in udder infections, milk has sometimes been examined for organisms of this type. Microscopic observations may be made, using milk direct or a sediment obtained by centrifuging, or cultural methods may be employed. With certain udder involvements the microscopic picture is quite characteristic because of the presence of long chain streptococci and large numbers of leucocytes but with others, also due to streptococci, the organisms are very inconspicuous and nothing characteristic is noted. The cultural method may consist of inoculating various quantities of milk into sugar bouillons and examining microscopically for streptococci or plates may be poured and colonies picked into some medium which is examined after growth has taken place.

The great variations in the pathogenicity and general characters of the streptococci suggest that little significance can be attached to the mere presence of this morphologic type. If there has been an opportunity for the growth of organisms in the milk, *S. lactis* and other typical milk streptococci may be numerous. Various investigators, such as Sherman and Hastings[37] and Evans,[38] have shown that streptococci are present in normal udders so that, even in fresh milk of a high quality, these organisms may be expected. It is probable that the long chain type coming from certain abnormal udders is non-pathogenic for man and so is comparatively unimportant, although the fact that the udder is diseased makes the milk objectionable.

---

[35] U. S. D. A. Bul. 739. 1918.

[36] Jr. Bact. 13. P. 419. 1927.

In some instances large numbers of streptococci have been found in cow feces and this has suggested the possibility of detecting heavy fecal contamination by examinations of milk for such organisms. The other sources from which streptococci may come greatly limit the conclusions that can be drawn.

It seems probable that a definite significance can be attached to the presence of streptococci in milk only when an identification of the organisms is made. The examination for types that are harmful will be of only limited value until methods are developed which make possible the detection of such organisms in a varied flora.

TESTS FOR SPECIFIC PATHOGENS

The detection of a pathogenic organism in milk provides the most definite type of information with reference to a milk supply. The methods of examination for certain of the pathogens, notably *M. tuberculosis*, yield accurate results but the methods for others are so unsatisfactory that negative findings do not admit of conclusions. The methods that are accurate are limited in their usefulness by the long periods required before the results are available. Because of the number of pathogenic species that are spread through milk, an examination for each would involve an excessive amount of work. Accordingly, tests for specific pathogens are only occasionally used in a routine way.

[37] Creamery and Milk Plant Monthly. 3. P. 11. Feb., 1915.

[38] Jr. Inf. Dis. 18. P. 437. 1916.

INDEX

A

Acetic acid fermentation, 74
Acid, influence on *E. typhosa,* 203
Acid development, importance of in cheddar cheese, 409
Acidity of fresh milk, 22
    cause, 22
  of milk, changes, 22
  titratable and pH, 24
  titration of milk and cream, 446
Acidophilus milk, 350
Actinomyces bovis, 181
Actinomycosis, spread through milk, 181
Advanced lactation, effect on flavor, 65
Aerobacter, genus, 40
Aerobacter aerogenes, 39
Air contamination in dairy plants, 124
Alcohol test, 447
  conditions causing coagulation, 447
  modifications, 448
  uses, 448
Alcohols in cheddar cheese, 417
Alkali poisoning, 184
Anaerobic spore test, 456
Anthrax, spread through milk, 180
Aseptically drawn milk, excessive numbers of bacteria, 80
  micrococci, 85
  numbers of bacteria, 79
  pathogenic organisms, 87
  rod shaped bacteria, 86
  streptococci, 86
  types of organisms, 85
  unusual organisms, 86
Aspergillus glaucus, 298
Aspergillus repens, 298
Associative action, 19
  as a cause of ropiness, 47
Associative action, in camembert cheese slime, 439
    color fermentations, 70
    eye formation in Swiss cheese, 429
    flavor and aroma fermentations, 68
    foamy cream, 54
    gassy butter culture, 55
    gassy cottage cheese, 55

B

Bacillus albolactus, 59
  amarus, 293
  anthracis, 180
  calidolactis, 138
  cereus, 59, 290
  coagulans, 290
    heat resistance, 290
  ichthyosmius, 293, 390
  lactimorbi, 184
  megatherium, 290
  panis, 293
  putrificus, 431
  rudensis, 423
  simplex, 290
  subtilis, 59, 293
Bacteria in udder, 79
  effect of various treatments, 109
Bacterial content of bottles, 91
  of cans, 91
    cow manure, 98
    dirt on cows, 98
    stable air, 90
    utensils, 91
Bacterial counts on milk, 1
      types, 1
  on pasteurized milk, 4
  research, 16
      media, 17
      incubation, 17

- Bacterial counts, routine, 2
    - criticism, 5
    - methods, 6
    - significance, 2
- Bacterial standards for cream, 15
  - for milk, 14
    - dual, 14
- *Bacterium acidi-propionici a*, 428
    - *d*, 429
  - *paraviscosum*, 45
  - *viscosum*, 44, 49
    - growth temperatures, 45
  - *visco-symbioticum*, 47
- Benzoic acid as a milk preservative, 252
- Bitterness at 10° C. (50° F.), 136
  - due to advanced lactation, 65
  - following proteolysis, 61
  - in evaporated milk, 292
- Blood in milk, 72
- Blue fermentation, 70
  - milk due to non-bacterial causes, 71
- Borax as a preservative, 252
- Boric acid as a preservative, 252
- Bottle caps, contamination from, 103
  - fillers, contamination from, 102, 125
- Bottled milk, distribution of bacteria, 244
- Bottles, bacterial content, 91
- Bovine type *M. tuberculosis*, in fatal cases in children, 157
  - in human tuberculosis, 156
    - various conditions, 158
- *Brucella melitensis*, 170, 175
  - heat resistance in milk, 259
- Buddesized milk, 252
- Butter, bacterial content compared to that of cream, 360
  - bacteriology, 354
  - causes of undesirable flavors in fresh, 366
  - causes of undesirable flavors that develop, 367
  - changes in numbers of bacteria, 363
  - circles, treatment, 398
  - color changes due to organisms, 391
  - desirable flavors, 365
  - determination of bacteria, 354
    - of yeasts and molds, 355
  - flavors, cooked, 381
- Butter flavors, cowy, 382
    - developed on holding, 385
    - distinctly foreign, 385
    - due primarily to off flavors in cream, 382
      - plant methods, 381
    - feed, 382
    - fishy, 388
    - flat, 381
    - high acid, 382
    - malty or burnt, 383
    - metallic, 384
    - moldy or musty, 383
    - neutralizer, 381
    - old cream or stale, 383
    - rancid, 387
    - sour, 382
    - storage, 385
    - surface taint, 390
    - tallowy, 386
    - yeasty, 382
  - growth of molds, 398
  - liners, treatment, 398
  - moldy, 391
  - numbers of organisms, 358
  - preventing contamination with molds, 396
  - significance of contamination with molds, 358
  - sources of flavor, 365
    - of molds, 394
      - organisms, 357, 359
  - specific flavor defects, 380
  - tubs, treatment, 398
  - types of molding, 394
  - types of organisms in fresh, 360
    - in old, 364
  - undesirable flavors, 365
  - wrappers, treatment, 398
- Butter cultures, abnormal flavors, 332
  - acidity, 329
  - amount of inoculating material, 327
  - bacteriology, 320
  - commercial, 322
  - containers, 325
  - controlling temperatures, 328
  - destruction of organisms during drying, 323

Butter cultures, development of mother, 325
    new, 345
  history, 320
  influence of air supply, 335
    of holding after cooling, 336
      incubation temperature, 333
      irregular temperatures, 333
      length of ripening, 334
      milk used, 334
      pasteurization exposure, 335
      various factors, 333
  judging, 330
    conditions to observe, 332
  methods of transferring, 327
  molds, 398
  natural, 321
  organisms normally present, 336
  over ripening, 329
  pasteurization of milk, 326
  preparation of large volumes, 330
  propagation, 324
  quality of milk, 325, 332
  temperature for development, 326
  types of lactic acid produced, 343
  volatile acids produced, 343
Butter deterioration, bacterial action, 368
  chemical action, 369
  effect of carbonating, 378
  enzymatic action, 376
  factors involved, 367
  fat, 380
  general types of changes, 379
  influence of acid, 370
    of air, 369
      metals, 374
      neutralization of cream, 374
  lactose, 379
  protein, 379
  relationship of composition, 377
Buttermilk, 348
  bulgarian, 349
Butyric acid fermentation, 73
  importance, 73
  organisms, characters, 73
    distribution, 73

C

Camembert cheese, bacteriology, 436
  importance of molds, 439
  manufacture from pasteurized milk, 441
  numbers of bacteria, 440
  O. lactis, 438
  organisms other than *S. lactis,* 437
  proteolysis, 440
  slime formation, 438
  S. lactis, 436
  typical mold, 437
Cans, bacterial content, 91
  condition of those returned to farms, 126
Capsular material in ropy milk, 45, 46
Catalase, 284
  demonstration, 284
  distribution, 284
  importance, 285
Catenularia fuliginea, 298
Cells, body, in milk, 143
  compared to per cent fat, 148
  evening and morning milk, 147
  in colostrum milk, 148
  influence of breed and age of cow, 149
    clarification and separation, 151
    condition of udder, 149
    stage of lactation, 148
    machine and hand drawn milk, 149
  methods of determining, 143
  numbers in normal milk, 145
  variations in portions of milking, 146
  variations in quarters, 146
Cellular test for pasteurized milk, 151
Changes in numbers of bacteria in milk, 138
  pH of bouillon by organisms in milk, 450
Characteristics of milk abnormalities due to organisms, 76
  milk-borne epidemics, 187
Cheddar cheese, alcohols and esters, 417
  changes in volatile acidity, 416
    in numbers of bacteria, 407
    produced by lactobacilli, 412
  clarification of milk, 426

Cheddar cheese, color changes, 426
  defects due to organisms, 418
  Escherichia-Aerobacter group in gassy, 420
  galactase action, 414
  gassy, 419
  lack of proper acid development, 418
  lactic acid, 416
  lactobacilli, 410, 415
  L. casei, 411
  manufacture from pasteurized milk, 424
  moldy, 423
  off flavors, 421
  peptic digestion, 414
  pink color, 423
  protein decomposition, 413
  quality of milk, 418
  S. citrovorus, 413
  S. lactis, 408, 415
  S. paracitrovorus, 413
  succinic acid, 417
Cheese, bacteriology, 401
Cheeses, classification, 401
  determination of bacteria, 403
  distribution of organisms, 403
  general ripening agents, 402
  types of milk, 405
Chemical action of washing powders, 128
  sterilization, influence of organic matter, 127
    of dairy equipment, 126
    milking machines, 113
Cholera, examples of milk-borne, 228
  spread through milk, 228
Churns, cleaning, 397
Circles, treatment, 398
Citric acid, decomposition by associated organisms, 339
Cladosporium butyri, 387
Clarification, 244
  effect on keeping quality, 249
    on pathogens, 250
  influence on bacterial content, 246
    on body cells, 248
      dirt, 249
  of milk for cheddar cheese, 426
Clarification, value, 250
Clarifier slime, amount, 246
  bacterial content, 245
  body cell content, 245
  composition, 244
Clostridium botulinum, 236
  butyricum, 74, 443
  sporogenes, 457
  welchii, 430
Clumps of bacteria, breaking up by clarification, 246
    by filtering, 240
      freezing ice cream, 315
      homogenization, 103
Coats of cows, contamination from, 97
  reducing contamination, 116
  types of organisms, 99
Cocci, yellow, in pasteurized milk, 268
Color fermentations, 69
Colostrum milk, numbers of bacteria, 83
  body cells, 148
Contagious abortion, spread through milk, 173, 178
  organism, dangers to man, 175
    examining milk, 177
    in milk, 173
    relationship, 175
Contamination, effect of brushing animals just before milking, 89
    feeding animals just before milking, 88
  from bottles, 91
    bottle caps, 103
    bottle fillers, 102, 125
    cans, 91
    churns, 359
    clothing, 100
    coat of cow, 97
    coolers, 101
    employees of plant, 105
    equipment, influence of season, 103
    external sources, 87, 101
    flies, 100
    freezers, 311
    homogenizers, 102, 125
    interior of udder, 78
    milker, 100
    milking machines, 94

Contamination, from stable air, 87
    tops of milk bottles, 106
    utensils, 91, 112
  in the homes, 106
  of dairy products with pathogens, summary, 232
  of milk and cream, 78
  on producing farms, 87
  subsequent to production, 101
Coolers, care, 113
  contamination from, 101
Cooling after pasteurization, 263
Corynebacterium diphtheriae—see diphtheria organism
Cottage cheese, addition of cream, 443
  bacteriology, 441
  deterioration, 442
  importance of pasteurizing milk, 442
  preservation, 444
  sources of flavor, 441
Cow manure, bacterial content, 98
  solubility in milk, 99
Cows giving high count milk, 80, 109

D

Dadhi, 353
Destruction of infectious material in dairy products, 233
  organisms in milk preservation, 253
Diarrhea, infantile, causative organisms, 228
    influence of type of feeding, 229
    spread through milk, 228
Diastase, 280
Diphtheria, example of butter-borne, 224
  examples of milk-borne, 221
  importance of milk as a cause, 225
  spread through milk, 221
  organism, growth in milk, 226
    isolation from milk, 226
    longevity in dairy products, 226
    resistance to heat, 259
    sources in milk, 226
Direct method for body cells, 145
Dirt, in milk, 97
    methods of measuring, 98
  on cows, bacterial content, 98
Diseases, groups spread through milk, 155
  spread among farm animals, 155
    through milk, 153
      derivatives, 154
Doane-Buckley method for body cells, 144
Drying milk, effect on various organisms, 302
Dysentery, example of milk-borne, 209
  organisms, growth in milk, 210
      heat resistance in milk, 259
      longevity in dairy products, 210
      sources in milk, 210

E

Eberthella typhosa, germicidal action on, 131
  growth in milk, 202
  heat resistance in milk, 259
  influence of acid, 203
  longevity in dairy products, 204
  sources in milk, 205
Electrical treatment of milk, 277
Emmental cheese—see Swiss cheese
Enzymes, action of pasteurization on, 275
  influence of pasteurization on, 276
  of milk, 280
Enzymes and organisms, differentiation of changes due to, 280
Epidemics, milk-borne, characteristics, 187
    methods of tracing, 189
Escherichia, genus, 39
Escherichia-Aerobacter group, biochemical features, 41
  causing gas in evaporated milk, 291
    in sweetened condensed milk, 297
  division, 39
  from coats of cows, 99
  gas production, 55
  growth in milk, 40
  heat resistance, 267
  importance in dairy products, 41
  importance in ropy milk, 45
  in aseptically drawn milk, 87

- Escherichia-Aerobacter group, in gassy cheddar cheese, 420
    - cottage cheese, 442
  - in milking machines, 97
    - pasteurized milk, 458
    - udder infections, 182
  - isolation from dairy products, 42
  - optical form of lactic acid, 42
  - on tops of bottles of milk, 106
  - presence in milk, 40
  - sources in milk, 41
  - species important in dairying, 42
  - tests for, 457
- *Escherichia coli*, 39
  - *neapolitana*, 46
- Equipment, chemical sterilization, 126
  - drying, 125
  - steaming, 125
  - washing, 124
- Esters in cheddar cheese, 417
- Evaporated milk, bacteriology, 287
  - bitterness, 292
  - bulging due to non-bacterial causes, 292
  - bulk, 293
  - coagulation, 288
  - fishiness, 293
  - gas formation, 291
  - heat coagulation, 290
  - incubation, 288
  - sterilization exposures, 287
  - types of spoilage due to organisms, 288

F

- Factors limiting infection of persons from milk, 187
- Fermentation cycle, raw milk, 24
- Fermented milks, bacteriology, 348
  - holding, 353
- Filtering milk, action in removing dirt, 241
  - action on keeping quality of milk, 241
  - effect on types of organisms, 241
  - general influence, 238
  - influence on bacterial content, 238
- Filters, contamination from, 241
- Flavor and aroma fermentations, 62
  - control, 69
  - importance, 62
  - types of organisms causing, 67
- Flavoring materials for ice cream, bacteria, 310
- Flavors and aromas, due to absorption, 62
  - due to advanced lactation, 65
    - condition of cows, 65
    - foreign materials, 66
    - infected udders, 65
    - materials consumed by cows, 63
    - sunlight, 66
  - influence of temperature on development, 68
  - not due to organisms, 62
  - types produced by organisms, 67
- Flies, contamination from, 100
- Foam, bacterial content during pasteurization, 262
  - temperature during pasteurization, 262
- Foot and mouth disease, cases in man, 178
  - spread through milk, 178
    - through skim milk, 179
- Fore milk, bacterial content, 81
  - influence of elimination, 107
- Formaldehyde as a preservative, 252

G

- Galactans in ropy milk, 48
- Galactase, 281
  - demonstration, 281
  - distribution, 282
  - heat resistance, 282
  - importance, 282
  - in cheddar cheese, 414
- Gas formation, in butter cultures, 56
  - in cheddar cheese, 419
    - cottage cheese manufacture, 442
    - yeasty cream, 51
  - production by anaerobes, 57
    - by *S. citrovorus*, 341
      - *S. paracitrovorus*, 341
      - torulae, 52
- Gassy fermentation, 51, 137
  - importance, 51

Gelatin for ice cream, bacteria, 308
  determination of bacteria, 308
Germicidal action of milk, 129
  action of heat, 131
  causes, 132
  influence of temperature, 130
  variations, 131
Gioddu, 353
Goats, history of a shipment of infected, 172
  Malta fever in, 172
Growth of organisms in milk and cream, 129
Gums in ropy milk, 48

H

Homogenizers, contamination from, 102, 125
Humidity, influence on mold growth in butter, 399
Hydrogen peroxide as a preservative, 252

I

Ice cream, bacteria in materials used, 307
  bacteriology, 304
  -borne epidemics, 318
  changes in bacteria during freezing, 314
    during hardening, 315
      manufacture, 312
      storage, 316
  contamination from air, 312
    from employees, 311
      utensils and equipment, 311
    incident to manufacture, 310
  determination of bacteria, 304
  effect of softening on bacteria, 317
  manufacture with low bacterial count, 317
  numbers of bacteria, 305
  sources of bacteria, 307
  types of bacteria, 306
Ice cream mix, changes in bacteria during aging, 314
    during homogenizing, 314
      making, 312
      pasteurizing, 313
Infantile diarrhea—see diarrhea
Infants, development on pasteurized milk, 275

J

Johne's disease, spread through milk, 179

K

Keeping quality tests, for milk, 449
  in presence of an indicator, 449
Kefir, 351
Kumiss, 352

L

Lactase, 280
Lactic acid, action in souring of milk, 23
  characters, 21
  condition in sour milk, 24
  effect of associated organisms, 339
  importance in butter cultures, 344
  in cheddar cheese, 416
  optical form in sour milk, 22
  optical form produced by butter cultures, 343
    by organisms—see various organisms
    isomers, 21
  preservative action, 24
  transformation of lactose to, 21
Lactic acid fermentation, 20
  organisms, types of, 25
    injected into udder, 85
Lactobacilli, changes produced in cheddar cheese, 412
  growth in cheddar cheese, 410
  in kefir, 351
  types in cheddar cheese, 411
Lactobacillus acidophil-aerogenes, 32
  acidophilus, 35, 350
  bulgaricus, 35, 349
    in controlling gas in Swiss cheese, 431
  casei, 35
    heat resistance, 267
Lactobacillus group, 32
  bio-chemical features, 36
  cultural characters, 36

Lactobacillus group, differentiation, 38
  growth temperatures, 37, 38
  importance in dairy products, 33
  in cream held warm, 137
  isolation from dairy products, 34
  maximum acidity, 37
  morphology, 36
  optical form of lactic acid, 37, 38
  presence in dairy products, 33
  proteolysis, 37, 412
  ropiness due to, 46
  species important in dairying, 35
  sources in milk, 33
  surface tension, effect on, 38
Lactobacillus thermophilus, 138, 269
Lactose, transformation to lactic acid, 21
Leben, 353
Liners, treatment, 398
Lipase, 65, 283
  demonstration, 283
  heat resistance, 284
  importance, 284

M

Malta fever, distribution, 171
  example of milk-borne, 173
  in goats, 172
  significance in goats in United States, 173
  spread through milk, 170
  organism, relationship, 175
Mazun, 352
Methylene blue reduction test, 451
  cause of reduction, 452
  influence of factors on, 452
  value, 453
  variations in action of organisms, 453
Micrococci causing thickening in sweetened condensed milk, 298
  in aseptically drawn milk, 85
    butter, 364
    contaminated acidophilus milk, 350
    milking machines, 97
Micrococcus caseolyticus, 85
  luteus, 85
  melitensis, 170
Milk acidity—see acidity
Milk, acidophilus, 350
Milk, changes above 37° C. (98.6° F.), 137
    at 4.4° C. (40° F.), 136
      10° C. (50° F.), 136
      21.1° C. (70° F.), 137
      37° C. (98.6° F.), 137
    at various temperatures, 133
    when held frozen, 133
      just above freezing, 135
  derivatives for ice cream, bacteria, 308
  fermentations, 18
    mixed, 19
  grades, 15
  importance in causing diseases—see various diseases
  inspection, value, 4
Milker, contamination from, 100
  reducing contamination, 120
Milking machines, bacterial flora, 96
  care, 113
  chemical sterilization, 113
  contamination from, 94
  heat sterilization, 114
  molds, 97
  quality of milk secured, 97
  sources of bacteria, 95
  treatment with cold water, 116
Milk powder, bacteriology, 299
  changes in organisms, 302
  contamination, 301
  deterioration, 302
  determining bacteria, 299
  effect of drying on organisms, 302
  numbers of organisms, 300
Milk sickness, plants important, 185
  spread through milk, 184
  theories as to cause, 184
Molds in butter, 358
  growth, 398
  sources in butter, 394
  preventing entrance, 396
Molds in cheese—see various cheeses
  in milk, 24
  milking machines, 97
Mucins in ropy milk, 48
Mycobacterium tuberculosis, effect of sunlight, 164

Mycobacterium tuberculosis, examining butter, 166
    cheese, 168
    ice cream, 168
    milk, 161
  growth in milk, 164
  heat resistance in milk, 256
  in blood, 162
    butter, 165
    cheese, 166
    feces of cows, 163
    ice cream, 168
    milk, 159
    oleomargarine, 166
    saliva, 164
    udder infections, 182
    uterine discharges, 164
  infection of milk, 161

N

Numbers of organisms, coming from utensils, 91, 112
  in milk, from different quarters, 83
    influence of age of animals, 84
      of incomplete milking, 84
        stage of lactation, 83
        type of feed, 84
  in milk drawn aseptically, 79
      various portions of milking, 81
    milk derivatives—see various products
    stable air, 90
  required to produce definite changes in milk, 74

O

Oidium lactis in butter, 360, 387, 388, 394
    deterioration, 377
  in camembert cheese, 438, 440
    milking machines, 97
  salt tolerance, 433
Oxygen, influence on mold growth in butter, 399

P

Paratyphoid fever, examples of milk-borne, 208
  spread through milk, 207
Paratyphoid organisms, growth in milk, 209
  longevity in dairy products, 209
  sources in milk, 209
Pasteurization, action on milk enzymes, 275
  action on products of bacteria, 275
  advantages, 271
  bacteria developing during, 270
  bacteria surviving, 265, 274
  bacterial efficiency, 256
  basis for exposures, 255
  commercial exposures, 260
  cooling after, 263
  effect of increased holding, 264
  final package, 255
  flash, 254
  for cheesemaking—see various cheeses
  holding, 255
  importance of uniform heating, 262
  improvement in keeping quality of milk, 273
  increase, 264
  inefficient, 265
  influence on composition of milk, 276
    vitamines, 276
  objections that have been raised, 274
  of sour cream, 361
  origin, 254
  reduction in infant mortality, 272
  reduction in spread of diseases, 271
  types, 254
  use to cover up dirty milk, 275
Pasteurized milk, bacterial changes, 271
  cellular tests, 151
  development of infants, 275
  Escherichia-Aerobacter group, 458
Pathogens, contamination of dairy products with, summary, 232
  in aseptically drawn milk, 87
  tests for, 459
Pellicle formation, influence on heat resistance of organisms, 261
Penicillium camemberti, 437
  proteolysis by, 439
  var. rogeri, 437
Penicillium roqueforti, 433
  changes produced by, 433

Penicillium roqueforti, factors influencing growth, 436
  salt tolerance, 433, 436
  tolerance of low oxygen tension, 433
Peroxidase, 285
  demonstration, 285
  importance, 285
  in butter, 286
    demonstration, 286
  in milk powders, 286
  resistance to heat, 285
Pin-point colonies, causes, 269
  from pasteurized milk, 5, 268
Piping, care, 397
Plants, air contamination, 124
Plate counts, 6
  objections, 6
  compared to direct count, 13
Poisoning, alkali, 184
  from dairy products, 234
    example of, 235
Poliomyelitis, examples of milk-borne, 226
  spread through milk, 226
Preservation of milk and cream, 237
  for analytical purposes, 253
Preservatives, general effect on organisms in milk, 251
  used in milk, 252
Prevention of entrance of infectious material to dairy products, 233
  of growth of organisms, 250
    by low temperatures, 250
    preservatives, 251
  spread of diseases through dairy products, 233
Propionic acid fermentation, 74
  organisms in Swiss cheese, 428
Proteolysis, 60
  in milk held just above freezing, 136
  relationship to sweet curdling, 61
Pseudomonas cyanogenes, 70
  fluorescens, 387
  synxantha, 71
Pumps, care, 397

Q

Quality of milk and cream, tests for, 445

R

Rabies, spread through milk, 181
Rancidity, due to advanced lactation, 65
  in butter, 387
    milk powder, 303
    sweetened condensed milk, 298
Rayless goldenrod, 186
Red fermentation, 72
Reducing contamination of milk, 107
  in dairy plants, 124
  on producing farms, 107
Reductase, 280
Removal of organisms from milk, 237
Rennet, effect on distribution of organisms, 406
Restraining organisms in plate counts, 17
Ropiness, at 10° C. (50° F.), 136
  materials causing, 48
Ropy fermentation, 42
  controlling, 49
  due to associative action, 47
  importance, 43
  in whey, 47
  responsible organisms, 44, 46
Ropy milk, organisms, distribution, 50
    heat resistance, 49
    isolation, 50
    sources, 48, 94
    types, 44, 46
Ropy milk, uses, 51
Roquefort cheese, bacteria other than *S. lactis*, 432
  bacteriology, 431
  characteristic flavor, 434
  protein decomposition, 434
  slime, 435
  S. lactis, 432
  typical mold, 433

S

Salicylic acid as a preservative, 252
Salolase, 280
Salt, restraining action on molds in butter, 398
  storage, 398
Salty flavor due to advanced lactation, 65

Scarlet fever, examples of milk-borne, 219
  importance of milk as a cause, 220
  spread through milk, 218
  organism, longevity in dairy products, 220
    sources in milk, 220
Separation, 242
  distribution of organisms, 242
  influence on pathogens, 244
Separators, care, 113
  contamination, 93
  growth of organisms, 93
Separator slime, materials in, 242
Septic sore throat, examples of milk-borne, 211
  importance of milk as a cause, 217
  organisms, heat resistance in milk, 259
  responsible organisms, 217
  sources of organisms in milk, 218
  spread through milk, 210
Serratia marcescens, 72, 85, 387
Sherbets, bacterial content, 318
Slimy fermentation—see ropy fermentation
Small top pails, 118
Snakeroot white—see white snakeroot
Stable, influence on milk contamination, 120
Stable air, bacterial content, 90
  reducing contamination, 110
  types of organisms, 90
Staphylococci, in udder infections, 182
Staphylococcus aureus, 295
Staphylococcus cremoris-viscosi, 46, 48
Starters—see butter cultures
Sterilization of milking machines, 113
  utensils, 111
Stewart-Slack method for body cells, 144
Strainers, care, 113
  contamination from, 90, 241
Straining, action in removing dirt, 239
    on keeping qualities, 239
  general influence, 238
  influence on bacterial content, 238
Streptococci in aseptically drawn milk, 86
Streptococci in udder infections, 182
  resistance to heat, 260, 268
  tests for, 458
Streptococcus citrovorus, 338
  distribution, 342
  in cheddar cheese, 413
  isolation, 341
  relationship to metallic flavor, 385
    to other organisms, 345
  variations, 343
  volatile acids produced, 343
Streptococcus distendens, 291
Streptococcus kefir, 345, 413
Streptococcus lactis group, acids other than lactic produced, 30
  associative action in acid formation, 30
  bio-chemical characters, 28
  cultural characters, 27
  development in presence of formaldehyde, 252
  distribution, 31
  enzyme action in acid production, 29
  fermenting capacity of cells, 29
  from utensils, 93
  germicidal action on, 131
  growth temperatures, 28
  identification, 28
  importance, 31
  in associative action causing ropiness, 47
  in cheddar cheese, 408, 415
  in kefir, 351
  in milking machines, 97
  inoculation into udder, 31
  isolation, 31
  maximum acidity, 28
  morphology, 26
  optical form of lactic acid, 28
  pathogenicity, 32
  proportion in butter cultures, 344
  proteolysis, 28
  sources, 31
  var. anoxyphilus, 26
  var. hollandicus, 26, 46
  var. maltigenes, 26, 31, 67, 383, 421
  var. tardus, 26
Streptococcus liquefaciens, 59, 62, 67
Streptococcus mastitidis, 182

Streptococcus paracitrovorus, 338
  distribution, 342
  heat resistance, 267
  in cheddar cheese, 413
  isolation, 341
  relationship to metallic flavor, 385
    to other organisms, 345
  type of lactic acid produced, 343
  variations, 342
  volatile acids produced, 343
Streptococcus pyogenes, 32, 183
Streptococcus thermophilus, 26, 267
Stringy milk, 43
Succinic acid in cheddar cheese, 417
Sugar for ice cream, bacteria, 310
    determination of bacteria, 310
Sunlight, effect on *M. tuberculosis*, 164
Sweet curdling fermentation, 57
  causative organisms, 58
  importance, 57
Sweetened condensed milk, bacteriology, 294
  buttons, 298
  chemical action in spoilage, 298
  enzymatic action in spoilage, 298
  gas formation, 296
  organisms in normal, 295
  rancidity, 298
  restraining action of sugar, 294
    of milk solids, 295
  tallowiness, 299
  thickening, 297
  types of spoilage due to organisms, 296
Swiss cheese, abnormal flavors and aromas, 431
  bacteriology, 426
  defects due to organisms, 430
  eye formation, 427
  gassy, 430
    control, 431
  general morphologic groups, 426
  lack of eye formation, 430
  organisms responsible for eyes, 428
  protein decomposition, 427

T

Tallowiness in butter, 386
Tallowiness in milk, 66
  milk powder, 303
  sweetened condensed milk, 299
Temperature, influence on growth of bacteria in milk, 138
    on mold growth in butter, 399
Temperatures for holding milk and cream, 140
Tests for quality of milk and cream, 445
  involving detection of products of growth of organisms, 445
    presence of certain types of organisms, 455
    rate at which changes are produced by organisms, 449
Torula amara, 68, 422
  cremoris, 52, 382
    isolation, 55
  glutinis, 72
  lactis-condensi, 296
  sphaerica, 52, 382
    isolation, 55
Trembles—see milk sickness
Tremetol, 186
Tommsdorff method for body cells, 144
Tuberculosis, financial losses with cattle, 170
  introduction into a herd, 168
  spread through milk, 156
Tubs, importance of paraffining, 400
  treatment, 398
Typhoid carriers, detection, 207
  prevalence among dairy employees, 206
  relationship to infection of milk, 205
Typhoid fever, example of cheese-borne, 197
    of ice cream-borne, 198
  examples of butter-borne, 196
    of milk-borne, 191
  importance of milk as a cause, 199
  spread through milk, 191
  organism—see *Eberthella typhosa*

U

Udder, influence of clipping on bacterial content of milk, 121

Udder inflammations, epidemics, 182
    extent, 181
    seasonal variations, 182
    types of organisms responsible, 182
    variations in susceptibility of cows, 182
    injection of organisms, 85
Ultra-violet rays, effect on bacteria in milk, 279
Utensils, construction, 113
  growth of organisms, 93
  numbers of organisms, 91, 112
  proper care, 110
  types of organisms, 93

V

Vacuum lines as a cause of contamination, 96
Variations in organisms, 19
Vats, care, 397
Vitamines, influence of pasteurization, 276
Volatile acid fermentations, 72
  acids in cheddar cheese, 416

W

Washing powders, chemical action, 128
Wet milking, 100
White snakeroot, 185
  toxic constituent, 186
Wisconsin curd test, 455
Wrappers, treatment, 398

Y

Yeasts in butter, 358, 364
  in cottage cheese, 442
    gassy Swiss cheese, 430
    milk, 24
  pink, development in butter, 400
    in cottage cheese, 442
Yeasty cream, 52
  control, 54
Yellow fermentation, 71
  milk due to non-bacterial causes, 71
Yogurt, 352

9/3

KQ-658-088